Section A1 **Annex Point IIA, I.1.1 and 1.2**	**Applicant**	
1.1 Applicant	Name: Dow AgroSciences B.V. Address: 41, Prins Boudewijnlaan, B-2650Edegem Antwerpen, Belgium Telephone: [redacted] Fax number: [redacted] Contact: [redacted] E-mail address: [redacted]	
1.2 Manufacturer of Active Substance (if different)	[redacted]	
1.3 Manufacturer of Product(s) (if different) **1) Product 1 (Spinosad Fly Bait)**	[redacted]	

	Evaluation by Competent Authorities	
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted	
	EVALUATION BY RAPPORTEUR MEMBER STATE	
Date	27 Nov 2006	
Materials and methods	No comments	
Conclusion	-	
Reliability	-	
Acceptability	acceptable	
Remarks	-	
	COMMENTS FROM...	
Date	*Give date of comments submitted*	
Results and discussion	*Discuss additional relevant discrepancies referring to the (sub) heading numbers and to applicant's summary and conclusion.* *Discuss if deviating from view of rapporteur member state*	
Conclusion	*Discuss if deviating from view of rapporteur member state*	
Reliability	*Discuss if deviating from view of rapporteur member state*	
Acceptability	*Discuss if deviating from view of rapporteur member state*	
Remarks		

Section A2.10 **Annex Point IIA, II.2.10**	**Exposure data in conformity with Annex VIIA to Council Directive 92/32/EEC (OJ No L, 05.06.1992, p. 1) amending Council Directive 67/548/EEC**	
Subsection		**Official use only**
2.10.1 Human exposure towards active substance		
2.10.1.1 Production	The biocidal active substance spinosad is manufactured in the USA. According to the TNsG Data Requirements, Ch.2, 2.10 and 6.6, for products manufactured outside the European Union, no details on production need to be included.	
i) Description of process	See above	
ii) Workplace description	See above	
iii) Inhalation exposure	See above	
iv) Dermal exposure	See above	
2.10.1.2 Intended use(s)	GF-739 (spinosad fly bait) is intended to control flies (Musca domestica) in animal stables.	
1. Professional Users	Professional operator (farmer)	

Section A2.10 **Annex Point IIA, II.2.10**	**Exposure data in conformity with Annex VIIA to Council Directive 92/32/EEC (OJ No L, 05.06.1992, p. 1) amending Council Directive 67/548/EEC**	
i) Description of application process	GF-739 is a granular bait product containing the active substance spinosad. It is used to control flies in animal housing. GF-739 may be applied in a number of different ways: **Method** / **Application** / **quency*** 1. Scattered evenly in animal housing where flies gather (e.g. window sills, tops of walls, edges of walkways, etc.). — 500 g product/200 (0.025 g a.s — pply when gr me dusty, fou , consumed o to cleaning pr t after 3-5 we 2. Placed in bait stations or trays, which are positioned where flies gather. — 500g product/200 floor space minimum o bait stations (0.025 g a.s — pply when gr me dusty, fou , consumed o to cleaning pr t after 3-5 we 3. Sprinkled onto moistened hang-boards/cards, where flies gather. — 100 g produ of board; 25 boards/200 floor space 5 m^2 of boards/200 floor space (0.025 g a.s — pply when gr me dusty, fou , consumed o to cleaning pr t after 3-5 we 4. Diluted in water and sprayed where flies gather. — 500 g product/1.0 ter; 1 L of diluted product/200 (0.025 g a.s 5 g a.s./L) — pply when gr me dusty, fou , consumed o to cleaning pr t after 3-5 we 5. Diluted with water and painted onto surfaces in areas where flies gather. — 500 g product/0.5 1.0 L water /200 m^2 (0.025 g a.s 5 or 10 g a.s — pply when gr me dusty, fou , consumed o to cleaning pr t after 3-5 we Do not apply more than 5 treatment regimes of GF-739 per annum in the same structure for pest resistance management purposes.	
ii) Workplace description	See above	
iii) Inhalation exposure iv) Dermal exposure	Please refer to Doc. IIIB 6.6, point 6.6.1.2.	

Section A2.10 **Annex Point IIA, II.2.10**	**Exposure data in conformity with Annex VIIA to Council Directive 92/32/EEC (OJ No L, 05.06.1992, p. 1) amending Council Directive 67/548/EEC**	
2. Non-professional Users including the general public	The spinosad fly bait is not intended to be used by the general public; the only user will be the professional farmer. GF-739 is a granular insecticide used in farm buildings. Therefore, since such premises are not occupied exposure of residents is not considered. Individual workers may enter treated premises for routine tasks and could come into contact with surface deposits of GF-739. However, exposure of such workers will be limited and can be expected to be considerably lower than those who applied the product. The calculations above demonstrate that exposure of operators to spinosad is below the AOEL for operators not wearing protective gloves. Therefore, the risk to other workers is considered to be very low.	
(i) via inhalation contact	Inhalation exposure arising from application of GF-739 would be negligible either applied as a granule or after spray dry. Spinosad is a non-volatile active substance (vapour pressure 2.0 - 3.0 x 10^{-11} kPa at 25°C, IIIA 3.2, Ref. A01 and A36) and GF-739 is a non-dusty granule (94% particles > 250 µm, Ref. IIIB 3.11/01, MA46.	
(ii) via skin contact	The impact of contact with the treated surface can be summarized in the following table. This is based on work by Brouwer *et al* 1999 and assumes transfer from hands to mouth and therefore absorption via the oral route. **Child** / **Adult** spinosad residue on treated surface (mg cm^{-2}): 0.0025 / 0.0025 dislodgeability of residues from treated surface = 2 % (mg cm^{-2}): 0.0001 / 0.0001 40 % of area of both palms contaminated (cm^2): 113.4 / 168 amount of spinosad residue on both palms = amount of spinosad ingested (mg): 0.0113 / 0.0168 body weight (kg): 36.3 / 60 Systemic exposure to spinosad *via* oral route (mg kg^{-1}): 0.0003 / 0.00028 % of AOEL: **1.25** / **1.17** Direct absorption via the skin would be even lower because of the lower efficiency of absorption via the skin.	
(iii) via drinking water	Not applicable, the baiting process does not involve application methods that will result in drift or off site movement or leaching.	
(iv) via food	Not applicable, since spinosad will not be used on food or feed. Indirect exposure via food is addressed in Section B6.7.	
(v) indirect via environment	Not applicable: If the product is used correctly and disposed of properly there should be no indirect exposure to the environment because the bait is only used inside of structures (animal housing/stables).	

Section A2.10 Annex Point IIA, II.2.10	Exposure data in conformity with Annex VIIA to Council Directive 92/32/EEC (OJ No L, 05.06.1992, p. 1) amending Council Directive 67/548/EEC	
	However, concern was raised during the national evaluation of this product about possible contamination of manure. Care should be taken that the product does not come into contact with manure for efficacy reasons. If spent fly bait granules are disposed of on manure heaps, the likelihood of residues of spinosad leaching out of the compost are extremely small due to the extremely high sorption constants for spinosad and its primary metabolites (Saunders, D.G. & Powers, F.L.(1994), Soil Adsorption and Desorption of XDE-105 Metabolite Factor B, DowElanco Report No. GH-C 3295, 91/414/EEC, IIA7.1.2/03 **Ref. K10** and Saunders, D.G. & Powers, F.L.(1994), Soil Adsorption and Desorption of XDE-105 Factor A, DowElanco Report No. GH-C 3298, 91/414/EEC, IIA7.1.2/02 **Ref. K07**). The range of Koc values for spinosyn A were 900-139698 mL/g. and for the primary metabolite, spinosyn B were 676-77418 mL/g These values indicate a very low potential for leaching out of manure. No specific study has been conducted with the Flybait product in manure, but there is a study available with the active ingredient Spinosad in a sediment under anaerobic conditions (Reeves, G.L., (1993), The Anaerobic Aquatic Metabolism of [14C] XDE-105, Dow AgroSciences report No.GHE-P-2989R, 91/414/EEC, IIA7.1.1.1.2/01, **Ref. K13**). This study is considered relevant to manure because anerobic conditions will occur in manure when it is wet. Once it dries out it would be aerobic. Therefore anaerobic metabolism is best studied under wet (aquatic) conditions. Under flooded anaerobic conditions in a sediment, spinosyns A and D partitioned rapidly from the water to sediment phases and, therefore, would be expected to remain strongly sorbed to the granular support material of the fly bait. Up to six metabolites were observed for spinosyn A and three of these exceeded 10% AR. At least eight metabolites were observed for spinosyn D and only one of these exceeded 10% AR. The HPLC profiles for spinosyn D extracts mirrored those for spinosyn A and the metabolites of spinosyn D were indicated to be the corresponding spinosyn A analogues. The major degradation products of spinosyn A (those exceeding 10% AR) were identified as spinosyn B, spinosyn J and a tentative structure of O-demethylated spinosyn J was proposed for the third major metabolite. Three minor metabolites (<10% AR) of spinosyn A were postulated to be isomers of the reversepseudoaglycone and ketoreversepseudoaglycone. The only metabolite of spinosyn D to exceed 10% AR was tentatively assigned as the spinosyn J analogue of spinosyn D (by analogy with spinosyn A). However, as spinosyn D is the minor component of spinosad (*ca.* 15-50%), this metabolite will not exceed 10% of the total applied spinosad. Levels of non-extractable residues in sediment reached maximum levels of 15- 17% AR under flooded anaerobic conditions. Production of volatile radioactivity was also low (<1% AR) for both spinosyns.	

Section A2.10 **Annex Point IIA, II.2.10**	**Exposure data in conformity with Annex VIIA to Council Directive 92/32/EEC (OJ No L, 05.06.1992, p. 1) amending Council Directive 67/548/EEC**	
	Conclusion: The likelihood of indirect exposure to the environment from use of the fly bait granule is extremely low because the bait is only used inside of structures (animal housing/stables). Concern has been expressed over the possible contamination of manure by the disposal of spent granules. Due to the extremely high sorption constant for spinosyn A and its primary metabolites, the risk of leaching of residues from manure is extremely low. Furthermore, under anaerobic conditions, residues of spinosad degrade to between 6 and 8 metabolites of similar structure to the parent materials, which will also have extremely low potential to leach from manure.	
2.10.2 Environmental exposure towards active substance		
2.10.2.1 Production	The biocidal active ingredient is manufactured in the USA. According to the TNsG Data Requirements, Ch.2, 2.10 and 6.6, for products manufactured outside the European Union, no details on production need to be included.	
(i) Releases into water	See above	
(ii) Releases into air		
(iii) Waste disposal		
2.10.2.2 Intended use(s)	Intended use is in product type 18 (insecticide) only.	
Predicted concentration in the affected compartment(s):	Air, water, soil and sediment were calculated according to the Fugacity Model Level I and Level II (refer to Document B7.8). The distribution of spinosad in the environment after use of GF-739 fly bait granule has been assessed using Level I and II fugacity models simulating an environment representative of the EU plus Norway and Switzerland. Based on the results of the modelling, the majority of spinosad released into the environment was predicted to be in the soil compartment (~99 %) and small amounts partitioned to the sediment (~1 %) and air, water, suspended solids, aerosol and fish compartments (<0.04 % in each). Total system fugacity values were in the range 1.62 x 10^{-17} Pa to 9.62 x 10^{-22} Pa for both Levels I and II. Equilibrium concentrations estimated at both Levels I and II were low for all compartments (<10^{-3} mg/kg). The results show that spinosad is unlikely to be found in any compartment at significant concentrations.	
water	<0.04 % in each	
sediment	~1 %	
air	<0.04 % in each	
soil	~99 %	

Section A2.10 Annex Point IIA, II.2.10	Exposure data in conformity with Annex VIIA to Council Directive 92/32/EEC (OJ No L, 05.06.1992, p. 1) amending Council Directive 67/548/EEC	
	Evaluation by Competent Authorities	
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted	
	EVALUATION BY RAPPORTEUR MEMBER STATE	
Date	12 January 2007	
Materials and methods	Section 2.10.1 (Human exposure towards active substance) Please refer to comments on Doc IIIB 6.6 Section 2.10.1 (v) Indirect exposure via the environment and section 2.10.2 Environmental exposure Exposure of water and soil via application of manure is the main relevant route as considered in the Emission Scenario Document for Pt 18. RMS has performed calculations for emissions to the environment according to the ESD, using all relevant information of the DAR. The results are presented in Doc IIB.	
Conclusion		
Reliability	see above	
Acceptability	see above	
Remarks		
	COMMENTS FROM...	
Date	Give date of comments submitted	
Results and discussion	Discuss additional relevant discrepancies referring to the (sub) heading numbers and to applicant's summary and conclusion. Discuss if deviating from view of rapporteur member state	
Conclusion	Discuss if deviating from view of rapporteur member state	
Reliability	Discuss if deviating from view of rapporteur member state	
Acceptability	Discuss if deviating from view of rapporteur member state	
Remarks		

Table A2.10.1: Workplace exposure / Inhalation exposure

Exposure scenario	Workplace operation	PPE	Year(s) of measurement	Number of measurements	Type of measurements	Exposure concentration
Production	not applicable, since spinosad is produced [redacted]					
Formulation	Formulation of the bait at the plant	Extensive engineering controls are in place to control exposure. Occasional use of PPE may be necessary if an environment becomes dusty.	No specific inhalation exposure measurements have been made for Spinosad. The health of the workforce is monitored regularly and no adverse effects associated with Spinosad have been reported.	-/-	-/-	-/-
Application MG 3 / PT18						
	1. Scattered evenly in animal housing where flies gather (e.g.	None required but gloves	None	None	None	The potential exposure was estimated using the most appropriate model available (US PHED) and an

Exposure scenario	Workplace operation	PPE	Year(s) of measurement	Number of measurements	Type of measurements	Exposure concentration
	window sills, tops of walls, edges of walkways, etc.). 500 g product/200 m^2 0.025 g a.s./m^2)	advised to avoid dyeing of hands				exposure of 1.2% of the AOEL was derived. This exposure would be further reduced as the operator would be recommended to wear gloves to avoid potential staining of the hands.
	2. Placed in bait stations or trays, which are positioned where flies gather. 500g product/200 m^2 floor space in a minimum of 10 bait stations (0.025 g a.s./m^2)	None required but gloves advised to avoid dyeing of hands	None	None	None	For placing undiluted product in bait stations/trays and when scattering by hand on to hangboards/cards, it can be anticipated that operator exposure to SpY granules will be far less than for scattering by hand directly to animal house surfaces. Also, it is likely that the duration of exposure will be less. Therefore, it is proposed that total systemic exposure for a 60 kg adult placing the product in bait stations/trays or on to hangboards/card will be **< 0.0003 mg kg^{-1} d^{-1}. i.e <1.2% of the AOEL.**
	3. Sprinkled onto moistened hang-boards/cards, where flies gather. 100 g product/m^2 of board; 25 boards/200 m^2 floor space using 5 m^2 of boards/200 m^2 floor space (0.025 g a.s./m^2)	None required but gloves advised to avoid dyeing of hands	None	None	None	For placing undiluted product in bait stations/trays and when scattering by hand on to hangboards/cards, it can be anticipated that operator exposure to SpY granules will be far less than for scattering by hand directly to animal house surfaces. Also, it is likely that the duration of exposure will be less. Therefore, it is proposed that total systemic exposure for a 60 kg adult placing the product in bait stations/trays or on to hangboards/card will be **< 0.0003 mg kg^{-1} d^{-1}. i.e <1.2% of the AOEL.**
	4. Diluted in water and sprayed where flies gather. 500 g	Gloves and FFP2 dust and	None	None	None	The exposure for a professional operator during mixing and application of GF-739 is calculated using 'spraying

Exposure scenario	Workplace operation	PPE	Year(s) of measurement	Number of measurements	Type of measurements	Exposure concentration
	product/1.0 L water; 1 L of diluted product/200 m^2 (0.025 g a.s./m^2; 5 g a.s./L)	liquid aerosol mask.				model 2' as presented in 'Assessment of human exposure to biocides'[1]. The model estimates the total exposure for mixing/loading and applying liquid remedial timber treatments and surface biocides by pumped sprayer and lance at 4 to 7 bar as a coarse spray indoors, overhead and downwards, and pressure-irrigating masonry. **The 75th percentile is considered a conservative value. Higher percentiles or maxima are not appropriate because The top end of the model represents contamination rates arising from use of high pressure hosing of large areas and the operators became visibly drenched over a period of time. It is difficult to imagine these higher rates of contamination arising from this process.** The potential exposure was estimated using the most appropriate model and an exposure of 57% of the AOEL was derived using gloves and FFP2 mask.
	5. Diluted with water and painted onto surfaces in areas where flies gather. 500 g product/0.5 L to 1.0 L water; /200 m^2 (0.025 g a.s./m^2; 5 or 10 g a.s./L)	None required but gloves advised to avoid dyeing of hands				Utilizing the Brush painting (includes decanting) from the draft is guidance includes the concepts developed in the report of the Biocides Steering Group (97/505/3040/DEB/E2) and refers to guidance on exposure assessment being developed for New and Existing Substances (NESS) 2002. The potential exposure was estimated using the most appropriate model available and an exposure of 27% of the AOEL was derived.

[1] Report to DGXI from the Biocides Steering Group, October 1998. Project 97/505/3040/DEB/E2. Page E.6.

Exposure scenario	Workplace operation	PPE	Year(s) of measurement	Number of measurements	Type of measurements	Exposure concentration
						However, the following facts indicate that this would be a conservative estimate;- ▪ No appropriate model exists for the use of a roller, but it is not unreasonable to deduce that the potential for dermal exposure during application would be less that that from the use of a brush. ▪ Exposure from mixing and loading a GF-739 would be low, in particular inhalation exposure as this formulation is a non-dusty granule (94% particles > 250 µm). The amount of formulation handled would be low (500 g product/200m^2) and the size of the granules would result in low retention on the skin. ▪ Inhalation exposure arising from application of GF-739 as a paint would be negligible as spinosad is a non-volatile active substance (vapour pressure 2.0 - 3.0 x 10^{-11} kPa at 25°C, IIIA 3.2, Ref. A01 and A36) and GF-739 is a non-dusty granule (94% particles > 250 µm, Ref. IIIB 3.11/01, MA46. ▪ Therefore the % AOEL based solely on dermal exposure would be 0.35/60 =0.0058mg/kg/day or 24%. of the AOEL. *This would further be reduced by the use of gloves*

Section A2 Annex Point IIA, II.2.1 to 2.7 and 2.9	Identity of Active Substance	
Subsection		Official use only

The document III A2.1 to 2.7 and 2.9 contains confidential information; therefore please refer to the CONFIDENTIAL folder.

Section A2.8 Annex Point IIA, II.2.8	Identity of impurities and additives (active substance)	

The document III A2.8 contains confidential information; therefore please refer to the CONFIDENTIAL folder.

As described in the "Guidance Document on How to utilize PPP dossiers/monograph" of 21 November 2003 the evaluation of the CA from the PPP 91/414/EEC monograph (Vol. 3 / Annex B) (the DAR) is used.

The full DAR (and its addenda) are enclosed in the "other documentation" section as described Document I.1 – Application form, point 6.9.

Below is an unchanged copy of the relevant parts of the Spinosad 91/414/EC Monograph written by the CA (CTB) and released in February 2001. No further information is given in the addenda to the draft assessment report of June 2002 and May 2005. Numbering as in the Monograph remains unchanged. 98/8/EC Annex numbering is included *in italic and shaded* for better referencing.

Table B.2.1 Summary of the physical and chemical properties of the active substance (studies were completed to an acceptable standard and results were considered to be valid unless specified otherwise)

section (Annex point)	study	purity	method	results	comment	reference
B.2.1.1 (IIA 2.1) ***IIIA 3.1.1***[2]	Melting point	98.3% (A) 98.0 %(D)	OECD No. 102 EEC Method A1	84 to 99.5°C 161.5 to 170.0 °C		Jones-Jefferson,1994
B.2.1.2 (IIA 2.1) ***IIIA 3.1.2***	Boiling point	pure	EEC method A 2	Not required for high melting point solids Measure up to 360°C	GLP	
B.2.1.3 (IIA 2.1) ***IIIA 3.10***	Temperature of decomposition or sublimation	88.0% (A+D)	In house methodno standard method DTA/DSC	92% wt loss during heating to 400°C Required if bp or mp cannot be determined due to decomposition or sublimation		Froelicher,1997

[2] Numbers in italic and grey shaded are 98/8/EC numbering included for ease of referencing.

section (Annex point)	study	purity	method	results	comment	reference
			acceptable			
CTB Comments at Completeness Check March 2006: Reference is made to the study of the determination of the decomposition temperature. The identity of relevant breakdown products is not determined in this study. A statement or study is necessary. Dow AgroSciences Response March 2006: The study (DAS Ref. A18) entitled "Thermogravimetric Analysis of Spinosad and Evolved Gas Analysis by Gas Chromatography/Mass Spectrometry" DECO GL-AL 96-005553, Froelicher, S.W, Feb 1997 has been submitted. This study provides details of the type of substances evolved from spinosad TGAI after heating to high temperatures > 400 deg C. Data from this thermogravimetric analysis indicated a sample weight loss of about 92% from the sample up to a temperature of 400 deg. Thermal degradation products associated with this weight loss tentatively identified by TG/GC/MS included Carbon dioxide, methyl formate, acetaldehyde, and various sugar fragments of the Spinosad molecule.						
B.2.1.4 (IIA 2.2) ***IIIA 3.1.3***	Relative density	88.0% (A+D)	OECD No. 109 EEC Method A3 Pyknometer method	0.512 at 20 °C		Jones-Jefferson,1994
B.2.1.5 (IIA 2.3) ***IIIA 3.2***	Vapour pressure	99.9% (A) >99 % (D)	OECD No. 104 EEC Method A4 Knudsen-Effusion/Weight Loss Method	A: 3.0×10^{-11} kPa at 25°C D: 2.0×10^{-11} kPa at 25°C		Chakrabarti,1991a and 1991b
B.2.1.6 (IIA 2.3) ***IIIA 3.2.1***	Volatility, Henry's law constant	pure	Calculation	A: 1.89×10^{-7} Pa $m^3 mol^{-1}$ D: 2.32×10^{-5} Pa $m^3 mol^{-1}$ solids or liquids determined or calculated from water	GLP <1E-5 very slightly volatile 1E-5-0.03 moderately volatile	Portwood, 1998a

section (Annex point)	study	purity	method	results	comment	reference
				solubility and vp (units Pa m^3 mol^{-1})	>0.03 highly volatile	
B.2.1.7 (IIA 2.4) ***IIIA 3.3.1***	Appearance: physical state	88.0% (A+D)	Visual Observation	light grey-white solid		Jones-Jefferson,1994
B.2.1.8 (IIA 2.4) ***IIIA 3.3.2***	Appearance: colour	88.0% (A+D)	Visual Observation	light grey-white solid		Jones-Jefferson,1994
B.2.1.9 (IIA 2.4) ***IIIA 3.3.3***	Appearance: odour	88.0% (A+D)		Slightly stale water		Jones-Jefferson,1994
B.2.1.10 (IIA 2.5) ***IIIA 3.4***	Spectra	95.0% (A) 95.6 % (D)	OECD No. 101	A: UV-spectrum, solution in methanol ε ($mol^{-1}cm^{-1}$) @ 244.2nm = $1.08x10^5$ ε ($mol^{-1}cm^{-1}$) @ 200.2nm = $5.73x10^4$ ε ($mol^{-1}cm^{-1}$) @ 244.0nm = $1.09x10^5$ ε ($mol^{-1}cm^{-1}$) @ 243.2nm = $1.10x10^5$ ε ($mol^{-1}cm^{-1}$) @ 201.0nm = $6.77x10^4$ D: solution in methanol ε ($mol^{-1}cm^{-1}$) @ 243.8nm = $1.10x10^5$ ε ($mol^{-1}cm^{-1}$) @ 202.8nm = $9.88x10^4$ ε ($mol^{-1}cm^{-1}$) @ 243.6nm = $1.10x10^5$ ε ($mol^{-1}cm^{-1}$) @ 242.6nm = $1.10x10^5$ ε ($mol^{-1}cm^{-1}$) @ 203.0nm = $1.08x10^5$ IR, NMR and MS-spectra were provided table of signal characteristics for interpretation UV/vis, IR, NMR, MS including molar extinction at relevant wavelengths. Mention any absorbance >290nm. Optical purity must be measured and specified for resolved optical isomers.	From the measurements submitted: the first two values for each spinosyn were measured in acidic methanol, the next value in basic methanol and the last two in neat methanol. Absorption at 290 nm: A: ε ($mol^{-1}cm^{-1}$) = $2.41 x10^2$ D: ε ($mol^{-1}cm^{-1}$) = $1.16x10^2$ GLP	Knowles, 1996 Hamilton et al, 1998a and 1998b

section (Annex point)	study	purity	method	results	comment	reference
				Spectra of tox., ecotox. or environmental significant impurities also required		
B.2.1.11 (IIA 2.6) ***IIIA 3.5***	Solubility in water	98.3% (A) and 99.9 % 99.8 % (D)	OECD No. 105 Flask method/Column elution Column elution	A: flask method and column elution: At 20°C: pH (distilled water) 89.4 mg/l, pH5: 290 mg/l, pH 7: 235 mg/l and at pH 9 16 mg/l (by column elution method D: only column elution: At 20°C water (pH 8.36): 0.495 mg/l at pH 5: 28.7 mg/l, at pH 7: 0.331 mg/l and at pH 9: 0.053 mg/l		Jones-Jefferson,1994 and Heimerl, 1993 and 1994
B.2.1.12 (IIA 2.7) ***IIIA 3.7***	Solubility in organic solvents (technical active substance)	98.3 % (A) 90.9 % (A) 98.0% % (D) 91.8 % (D)manufactured	OECD 105 Shaking flask method EEC A6 Shaking flask method (underlined results) OECD 105 Shaking flask method EEC A6 Shaking flask method(underlined results)	A at 20°C in: dichoromethane: 525 g/l; methanol: 190 g/l; acetone: 168 g/l; acetonitrile: 134 g/l amyl acetate: 36.9 g/l; hexane: 4.48 g/l; 1-octanol: 9.26 g/l: tolene: 457 g/l and iso-propanol: 39.8 g/l A at 20°C in : ethyl acetate: 194; n-heptane: 12.4 g/l and xylene; > 250 g/l 15-25°C report of <250 g/kg ethyl acetate D at 20°C in: dichoromethane: 448 g/l; methanol: 2.52 g/l; acetone: 10.1 g/l; acetonitrile: 2.55 g/l; amyl acetate: 23 g/l; hexane: 0.743 g/l; 1-octanol: 1.27g/l; toluene: 152 g/l and iso-propanol: 1.29 g/l D at 20°C in : ethyl acetate: 19 g/l; n-heptane: 0.3 g/l and xylene: 64 g/l 15-25°C report of <250 g/kg ethyl acetate	<0.1 mg/l very slightly soluble 0.1-10 slightly soluble 10-1000 moderately soluble ≥1000 readily soluble	Jones-Jefferson,1994 And Comb, 1997a and 1997b

section (Annex point)	study	purity	method	results	comment	reference
B.2.1.13 (IIA 2.8) ***IIIA 3.9***	Partition co-efficient	97% (A) 98% (D)	EPA/FIFRA Subdivision D 63.11 Shake flask method	A: Log K_{ow} = 3.91 @ 23°C (water) Log K_{ow} = 2.78 @ 23°C (pH 5) Log K_{ow} = 4.01 @ 23°C (pH 7) Log K_{ow} = 5.16 @ 23°C (pH 9) D: Log K_{ow} = 4.38 @ 23°C (water) Log K_{ow} = 3.23 @ 23°C (pH 5) Log K_{ow} = 4.53 @ 23°C (pH 7) Log K_{ow} = 5.21 @ 23°C (pH 9)		Morrisey, 1994a and 1994b
B.2.1.14 (IIA 2.9) ***IIIA 7.1.1.1.1***	Stability in water	A: 94.7% radiochem. pure D: 93.6 radiochem. pure	FIFRA Guideline 161-1 Determined at 25 °C	A: At pH 5: no hydrolysis; at pH7, DT50 = 648 days and at pH9, DT 50 = 200 days; D: At pH 5 and 7 no hydrolysis; at pH 9, DT 50 = 259 days.	**1 NO SIGNIFICANT HYDROLYSIS FOR FACTOR A AND D AT PH 5 AND 7. FACTOR A AND D ARE STABLE TO HYDROLYSIS AT PH 5 AND 7 AT 25 ^{0}C.** **2 VERY SLIGHTLY HYDROLYSING AT PH 9.**	Saunders , Powers and Cooket all, 1994

section (Annex point)	study	purity	method	results	comment	reference
					3 GLP DT50 at 20°C, pH7 >30d slightly hydrolysing 10-30 moderately hydrolysing 4-10 fairly hydrolysing 1-4 readily hydrolysing <1 very rapidly hydrolysing	
B.2.1.15 (IIA 2.9) ***IIIA 7.1.1.1.1***	Hydrolysis rate	pure	EEC method C 7	see above hydrolysis rate at pH 4, 7 and 9 sterile conditions, absence of light low hydrolysis rate-determine at 50°C or other appropriate temp. If degradation at <50°C, determine at 20°C using Arrhenius plot	GLP	

section (Annex point)	study	purity	method	results	comment	reference
B.2.1.16 (IIA 2.9) ***IIIA 7.1.1.1.2***	Photochemical degradation	A: 94.7% radiochem. pure D: > 93.6 radiochem. pure	FIFRA Guideline No. 161-2	A: The half-life for the degradation of spinosyn A in dilute aqueous buffer was calculated to be 0.96 days, in summer sunlight (June-July in Greenfield, Indiana, 39.8°N). D: The half-life for the degradation of spinosyn D in dilute aqueous buffer was calculated to be 0.84 days, in summer sunlight (June-July in Greenfield, Indiana, 39.8°N).	Conditions: pH 7 and 25°C, natural sunlight was used. The concentration of acetonitrile was 0.5 % 1,2-14C-acetate is used as the carbon source and the 14C spinosad is produced by fermentation. The radiolabel is incorporated fairly uniformly throughout the macrolide ring and also on the first ethyl group carbon at position 21 and the methyl at position 16. No information is available , how many of the 23 available carbons are radiolabeled in a typical fermentation run. The main conclusion is that there are no	Saunders and Powers 1994

section (Annex point)	study	purity	method	results	comment	reference
					radiolabeled carbons on either sugar group.	
B.2.1.17 (IIA 2.9) ***IIIA***	Quantum yield	94.7% (A) > 93.6% (D)	FIFRA Guideline No. 161-2	0.019 (A) 0.021 (D)	See above	See above
B.2.1.18 (IIA 2.9) ***IIIA 3.6***	Dissociation constant (pKa)	97% (A) 97% (D)	OECD Guideline 112 Capillary electrophoresis method	pKa of protonated Factor A = 8.10 at 20° C, equivalent Ka = 7.94 x 10^{-9}. pKa of protonated Factor D = 7.87 at 20° C, equivalent Ka = 1.35 x 10^{-8}.	Protonation of the N-atom	Gluck, 1994a and 1994b
B.2.1.19 (IIA 2.10) ***IIIA 7.3.2***	Stability in air, photochemical oxidative degradation		Atmosperic Oxidation Program (Atkinson Calculation)	Spinosyn A Rate Constant 382.2 10^{-12} cm^3/molecule-sec Half Life 20.1 minutes (Hydroxyl Concentration 1.5x10^6)		Portwood, 1998

section (Annex point)	study	purity	method	results	comment	reference
				Spinosyn D Rate Constant 412.8 10^{-12} cm^3/molecule-sec Half Life 18.7 minutes (Hydroxyl Concentration 1.5x10^6)		
B.2.1.20 (IIA 2.11) ***IIIA 3.11***	Flammability and auto-flammability (technical active substance)	88.0% (A+D)	EEC Method A10 EEC Method A16	Not flammable None below 400°C		Sydney, 1997

CTB Comments at Completeness Check March 2006:

Reference is made to the study of the determination of the flammability and autoflammability (according to EEC method A10 and A16) The identity of combustion products is not determined in this study.
A statement or study is necessary.

Dow AgroSciences Response March 2006:

The flammability test on spinosad (EEC A10) indicated that the material was not flammable. The autoflammability test (EEC A16) on spinosad indicated that the material was not autoflammable and did not self-ignite even up to the maximum temperature of 400ºC. We therefore submit that the identity of combustion products from these 2 tests is not relevant or appropriate.

The study (DAS Ref. A18, submitted under 98/8/EC point IIIA3.10)) entitled "Thermogravimetric Analysis of Spinosad and Evolved Gas Analysis by Gas Chromatography/Mass Spectrometry" DECO GL-AL 96-005553, Froelicher, S.W, Feb 1997 has been submitted. This study provides details of the type of substances evolved from spinosad TGAI after heating to high temperatures > 400 deg C.
Data from this thermogravimetric analysis indicated a sample weight loss of about 92% from the sample up to a temperature of 400 deg. Thermal degradation products associated with this weight loss tentatively identified by TG/GC/MS included Carbon dioxide, methyl formate, acetaldehyde, and various sugar fragments of the Spinosad molecule.

section (Annex point)	study	purity	method	results	comment	reference
B.2.1.21 (IIA 2.12) ***IIIA 3.12***	Flash point (technical active substance)				Not required, melting point > 40°C	Sydney, 1997
B.2.1.22 (IIA 2.13) ***IIIA 3.15***	Explosive properties (technical active substance)	88.0% (A+D)	EEC Method A14 Koenen steel tube test	Not explosive		Sydney, 1997
B.2.1.23 (IIA 2.15) ***IIIA 3.16***	Oxidising properties (technical active substance)	88.0% (A+D)	EEC Method A17	Non-oxidising		Sydney, 1997
B.2.1.24 (IIA 2.14) ***IIIA 3.13***	Surface tension	90.9% (A)	EEC Method A5	A: 41.5mN/m D: EEC Method A5 states that the test is not necessary where water solubility < 1mg/L		Comb, 1999

B.2.3 Summary of physical and chemical properties

B.2.3.1 Active substance

Physical and chemical properties of the active substance

Spinosad is a mixture of two structurally similar molecules which are both active insecticidally and have been designated spinosyn A and spinosyn D. Spinosad typically contains Spinosyn A and spinosyn D in a ratio of approximately 85 % A : 15 % D.

The pure active substance spinosyn A and D are solids with a melting range from 84 to 99.5 ^{0}C for A and 161.5 to 170.0 ^{0}C for D. The vapour pressure is low for both isomers.

Spinosyd D is slightly soluble in water (0,33 mg/ml at pH7) while spinosyn A is moderately soluble in water (235 mg/l at pH 7) with some decrease at higher pH values. The log Pow is slightly pH dependable but values higher than 4 at pH 7 and higher pH values indicates that bio-accumulation can occur. Log Pow at pH 7 is for spinosyn A: 4.01 and for spinosyn D: 4.53.

The active substance is very slightly hydrolysing in water (at pH 9) but degradation during radiation with sunlight is very rapidly. The dissociation constant of spinosyn A is pKa is 8.1 and for D, pKa is 7.87.

The technical substance is not classified as flammable, auto-flammable, explosive or oxidising.

B.2.4 References relied on

References for the active substance

91/414/EC Annex point / reference no. No.	Author(s)	Year	Title Company Report No. Source (where different from company) GLP or GEP status Published or not	Data protection claimed Y/N	Owner
IIA 2.1.1/ 2.2/2.4.	Jones-Jefferson, T.J.	1994	Series 63: Physical and Chemical Characteristics of the technical grade of Active Ingredient XDE-105, Report no.: GH-C 3443 GLP study Not published	Y	DAS (A03)

91/414/EC Annex point / reference no. No.	Author(s)	Year	Title Company Report No. Source (where different from company) GLP or GEP status Published or not	Data protection claimed Y/N	Owner
IIA 2.1.3	Froelicher, S. W.	1997	Thermogravimetric Analysis of Spinosad and Evolved Gas Analysis by Gas Chromatography/Mass Spectrometry, Study no.: DECO GL-AL 96-005553 GLP study Not published	Y	DAS (A18)
IIA 2.3.1	Chakrabarti, A.	1991a	Vapour Pressure of Compound 232105 measured by the Knudsen-Effusion/Weight Loss Method Report no.:ML-AL-91-020220 GLP study Not published	Y	DAS (A01)
IIA 2.3.1	Chakrabarti, A.	1991b	Vapour Pressure of Compound 275043measured by the Knudsen-Effusion/Weight Loss Method Report no.: ML-AL-91-020221 GLP study Not published	Y	DAS (A36)
IIA 2.3.2	Portwood, D.	1998a	Determination of Henry's Law Constant for Spinosad Study no.: DCW 901/970497 GLP study Not published	Y	DAS (A46)

91/414/EC Annex point / reference no. No.	Author(s)	Year	Title Company Report No. Source (where different from company) GLP or GEP status Published or not	Data protection claimed Y/N	Owner
IIA 2.5.1	Hamilton, T., Babbit, G., & Castetter, S	1998a	Determination of the Purity and Identity of Spinosyn A Pure Active Ingredient, TSN 100941 Report no.: GH-C 4746 GLP study Non published	Y	DAS (A41)
IIA 2.5.1	Hamilton, T., Babbit, G., & Castetter, S	1998b	Determination of the Purity and Identity of Spinosyn D Pure Active Ingredient, TSN 100222 Report no.: GH-C 4744 GLP study Non published	Y	DAS (A42)
IIA 2.5.1	Knowles, S.	1996	Generation of UV-VIS Spectral Data for DE-105 Factor A TSN 1011599 and DE-105 factor D, TSN 10116000 Report no.: GHE-P-5674 GLP study Non published	Y	DAS (A15)
IIA 2.6	Heimerl, J. L.	1993	Solubility of Compound 232105 in pH=9 Buffer Solution for Registration Report no.: DECO ML-AL 92/080163 GLP study Not published	Y	DAS (A20)

91/414/EC Annex point / reference no. No.	Author(s)	Year	Title Company Report No. Source (where different from company) GLP or GEP status Published or not	Data protection claimed Y/N	Owner
IIA 2.6	Heimerl, J. L.	1994	Solubility of Compound 275043 in Water and Buffer solutions of pH= 5, 7 and 9 (for Registrations) Report no.: DECO ML-AL 94/280051 GLP study Not published	Y	DAS (A37)
IIA 2.7	Jones-Jefferson, T. J.	1994b	Determination of Solubility of XDE-105 Factor A Report no.: GH-C 3376 GLP study Not published	Y	DAS (A10)
IIA 2.7	Jones-Jefferson, T. J.	1994c	Determination of Solubility of XDE-105 Factor D Report no.: GH-C 3368 GLP study Not published	Y	DAS (A06)
IIA 2.8	Morrissey, M. A.	1994a	Octanol/Water Partition Coefficient Determination of Compound 232105 Report no.: GH-C 3299 GLP study Not published	Y	DAS (A08)
IIA 2.8	Morrissey, M. A.	1994b	Octanol/Water Partition Coefficient Determination of Compound 275043 Report no.: GH-C 3300 GLP study Not published	Y	DAS (A47)

91/414/EC Annex point / reference no. No.	Author(s)	Year	Title Company Report No. Source (where different from company) GLP or GEP status Published or not	Data protection claimed Y/N	Owner
IIA 2.9.1	Saunders, D. G., Powers, F. L.& Cook, W. L.	1994	Hydrolysis of XDE-105 Factors A and D in Aqueous Buffer Report no.: GH-C 3228 GLP study Not published	Y	DAS (K05)
IIA 2.9.2/ 2.9.3	Saunders, D. G., Powers, F. L	1994	Photodegradation of XDE-105 Factors A and D in pH 7 Buffer Report no.: GH-C 3044 GLP study Not published	Y	DAS (K06)
IIA 2.9.4	Gluck, S. J.	1994a	Determination of the Dissociation Constant of LY 232105 Report no.: ML-AL 93-0800500 GLP study Not published	Y	DAS (A04)
IIA 2.9.4	Gluck, S. J.	1994b	Determination of the Dissociation Constant of XDE-105 Factor D Report no.: ML-AL 93-080499 GLP study Not published	Y	DAS (A07)
IIA 2.10	Portwood, D.	1998	Estimation of Photochemical Oxidative Degradation of Spinosad Report no.: GHE-P-7104 GLP study Not published	Y	DAS (A38)

91/414/EC Annex point / reference no. No.	Author(s)	Year	Title Company Report No. Source (where different from company) GLP or GEP status Published or not	Data protection claimed Y/N	Owner
IIA 2.11.1/ 2.11.2/ 2.11.13/2.15	Sydney, P.	1997	Spinosad: Determination of the Physico-Chemical Properties Report no.: GHE-P-6475 GLP study Not published	Y	DAS (A34)
IIA 2.14	Comb, A. L.	1997a	Spinosad: Determination of the Physico-Chemical Properties-Surface Tension and Solvent Solubility Report no.: GHE-P-7782 GLP study Not published	Y	DAS (A44)
IIA 2.14	Comb, A. L.	1997b	Spinosad: Determination of the Physico-Chemical Properties-Surface Tension and Solvent Solubility Report no.: GHE-P-7781 GLP study Not published	Y	DAS (A45)

Further studies were conducted after the release of the 91/414/EC Draft Assessment report and are summarized below:

98/8/EEC Section A3	**Physical and Chemical Properties of Active Substance**							
Subsection Annex Point IIA, III.3.1 to 3.13 and IIIA, III0§, III.1 and III.2.	**Method**	**Purity/ Specifi -cation**	**Results** Give also data on test pressure, temperature, pH and concentration range if necessary	**Remarks/ Justification**	**GLP (Y/N)**	**Relia bility**	**Reference**	**Offici al use only**
3.1 Melting point, boiling point, relative density (IIA3.1)								
3.1.1 Melting point	See above							
Melting pt. 1								
3.1.2 Boiling point	See above							**X**
Boiling pt. 1								
3.1.3 Bulk density/ relative density	OECD No. 109 EEC Method A3 Pyknometer method	Spino syn A 90.9 %	1.1244 g/cm^3	none	Y	1	Ref. IIIA3.1. 3/01, A55 Karyn, Huntley and Lyn Edgar, 2000	
	OECD No. 109 EEC Method A3 Pyknometer method	Spino syn D 91.8 %	1.1686 g/cm^3		Y	1	Ref. IIIA3.1. 3/01, A55 Karyn,	

98/8/EEC Section A3	Physical and Chemical Properties of Active Substance							
Subsection Annex Point IIA, III.3.1 to 3.13 and IIIA, III0§, III.1 and III.2.	Method	Purity/ Specifi -cation	Results Give also data on test pressure, temperature, pH and concentration range if necessary	Remarks/ Justification	GLP (Y/N)	Relia bility	Reference	Offici al use only
							Huntley and Lyn Edgar, 2000	

98/8/EEC Section A3	Physical and Chemical Properties of Active Substance							
Subsection Annex Point IIA, III.3.1 to 3.13 and IIIA, III0§, III.1 and III.2.	Method	Purity/ Specifi -cation	Results Give also data on test pressure, temperature, pH and concentration range if necessary	Remarks/ Justification	GLP (Y/N)	Relia bility	Reference	Offici al use only

CTB Comments during Evaluation August 2007:

The relative density was determined in two different studies as 0.512 or 1.1866 at 20 °C. No explanation was given for this difference in results. However, in an addendum to the 91/414 DAR on pesticides, an explanation was given for this difference. The applicant is requested to update Doc IIIA to reflect the current status of the DAR.

Dow AgroSciences Response August 2007.

The Addendum to the 91/414/EEC Draft Assessment Report of July reflects the current studies conducted. A new study for the relative density was stated because the value in the original study (0.512 at20*C at 88.0% purity) was not correct. It was apparent that such a low value was inconsistent with what would be expected for a molecule of this molecular weight.

91/414 (Annex point)	study	purity	method	results	comment	reference
91/414 B.2.1.4 (IIA 2.2)	Relative density	88.0%	OECD 109 (pycnometer)	Density: 1.19 20°C)	GLP, acceptable	Huntley, 2000 (DAS ref. no. A55, 98/8 Ref IIIA3.1.3/01.) Huntley, K., Determination of color, physical state, odor and density Spinosyn A, Spinosyn D and Spinosad technical. ABC laboratories inc., Columbia, USA, 3 March 2000. Rep. No. 45885, GLP, not published

98/8/EEC Section A3	Physical and Chemical Properties of Active Substance							
Subsection Annex Point IIA, III.3.1 to 3.13 and IIIA, III0§, III.1 and III.2.	Method	Purity/ Specifi -cation	Results Give also data on test pressure, temperature, pH and concentration range if necessary	Remarks/ Justification	GLP (Y/N)	Relia bility	Reference	Offici al use only
3.2 Vapour pressure (IIA3.2)	See above							
Vapour pressure 1								
Vapour pressure 2								
3.2.1 Henry's Law Constant (Pt. I-A3.2)	See above							
3.3 Appearance (IIA3.3)	See above							
3.3.1 Physical state	See above							
3.3.2 Colour	Visual Observation ASTM Method D1535-89	Spino syn D 91.8 %	Hue of 5 Y, a value of 9 and a chroma of 1 at 23.4° C		Y	1	Ref. IIIA3.3. 2/01, A55 Karyn, Huntley and Lyn Edgar, 2000	

98/8/EEC Section A3	Physical and Chemical Properties of Active Substance							
Subsection Annex Point IIA, III.3.1 to 3.13 and IIIA, III0§, III.1 and III.2.	Method	Purity/ Specifi -cation	Results Give also data on test pressure, temperature, pH and concentration range if necessary	Remarks/ Justification	GLP (Y/N)	Relia bility	Reference	Offici al use only
	Visual Observation ASTM Method D1535-89	Spino sad techn ical	Value of N 9.25, and a percent reflectance of 84.2% at 23.5° C.		Y	1	Ref. IIIA3.3. 2/01, A55 Karyn, Huntley and Lyn Edgar, 2000	
3.3.3 Odour	Visual Observation	Spin osyn A 90.9 %	Fish-like, wax-like, and paint odour at 23.2° C.		Y	1	Ref. IIIA3.3. 3/01, A55 Karyn, Huntley and Lyn Edgar, 2000	
	Visual Observation	Spin osyn D 91.8 %	Paint-like, bitter, and aspirin-like at 23.2° C.		Y	1	Ref. IIIA3.3. 3/01, A55 Karyn, Huntley	

98/8/EEC Section A3	Physical and Chemical Properties of Active Substance							
Subsection Annex Point IIA, III.3.1 to 3.13 and IIIA, III0§, III.1 and III.2.	Method	Purity/ Specifi -cation	Results Give also data on test pressure, temperature, pH and concentration range if necessary	Remarks/ Justification	GLP (Y/N)	Relia bility	Reference	Offici al use only
							and Lyn Edgar, 2000	
	Visual Observation	Spin osad Tech nical	Chalk-like, dusty odour at 23.2° C		Y	1	Ref. IIIA3.3. 3/01, A55 Karyn, Huntley and Lyn Edgar, 2000	
3.4 Absorption spectra (IIA3.4)	See above							
UV/VIS	See above							
IR	See above							
NMR	See above							
MS	See above							

98/8/EEC Section A3	Physical and Chemical Properties of Active Substance							
Subsection Annex Point IIA, III.3.1 to 3.13 and IIIA, III0§, III.1 and III.2.	Method	Purity/ Specifi -cation	Results Give also data on test pressure, temperature, pH and concentration range if necessary	Remarks/ Justification	GLP (Y/N)	Relia bility	Reference	Offici al use only
3.5 Solubility in water (IIA3.5)	See above							
Water solubility 1								
3.6 Dissociation constant (-)	See above							
3.7 Solubility in organic solvents, including the effect of temperature on solubility (IIIA3.1)	See above							
3.8 Stability in organic solvents used in b.p. and identity of relevant breakdown products (IIIA3.2)			The active substance spinosad as manufactured does NOT include an organic solvent, therefore according to the TNsG, Ch.3/Part A such a test does not need to be performed.					
3.9 Partition coefficient n-octanol/water (IIA3.6)	See above							
log Pow 1								
3.10 Thermal stability,	See above							

98/8/EEC Section A3	Physical and Chemical Properties of Active Substance							
Subsection Annex Point IIA, III.3.1 to 3.13 and IIIA, III0§, III.1 and III.2.	Method	Purity/ Specifi -cation	Results Give also data on test pressure, temperature, pH and concentration range if necessary	Remarks/ Justification	GLP (Y/N)	Relia bility	Reference	Offici al use only
identity of relevant breakdown products (IIA3.7)								
3.11 Flammability, including auto-flammability and identity of combustion products (IIA3.8)	See above							
3.12 Flash-point (IIA3.9)	See above							
3.13 Surface tension (IIA3.10)	See above							
3.14 Viscosity (-)				This data is always required for liquid substances. Spinosad is a solid.				
3.15 Explosive properties (IIA3.11)	See above							
3.16 Oxidizing properties (IIA3.12)	See above							
3.17 Reactivity towards	Dow AgroSciences	89.6	The post storage analysis of spinosad technical	-/-	Y	1	98/8	

98/8/EEC Section A3	Physical and Chemical Properties of Active Substance							
Subsection Annex Point IIA, III.3.1 to 3.13 and IIIA, III0§, III.1 and III.2.	Method	Purity/ Specifi -cation	Results Give also data on test pressure, temperature, pH and concentration range if necessary	Remarks/ Justification	GLP (Y/N)	Relia bility	Reference	Offici al use only
container material (IIA3.13)	Standard Operating Procedure FST-52	%	stored in flexible polypropylene bags showed no change in active ingredient content. An evaluation of the analytical data against EPA certified limits criteria shows that spinosad technical can be stored in excess of one year under typical warehouse conditions in flexible polypropylene bags without exceeding the EPA Certified Limits for the product. The stored spinosad technical showed no changes in colour or consistency during storage. Weight changes in the packaged product were insignificant and no package deterioration was observed during the study.				A 3.17/01, A58	

Reference used:

98/8 Annex point / reference no. No.	Author(s)	Year	Title Company Report No. Source (where different from company) GLP or GEP status Published or not	Data protection claimed Y/N	Owner

98/8 Annex point / reference no. No.	Author(s)	Year	Title Company Report No. Source (where different from company) GLP or GEP status Published or not	Data protection claimed Y/N	Owner
A3.1.3/01, Ref. A55 A3.3.2/01, Ref. A55 A3.3.3/01, Ref. A55	Karyn, Huntley and Lyn Edgar, 2000	2000	Determination of Color, Physical State, Odor, and Density for Spinosyn A, and Spinosyn D, and Spinosad Technical	Y	DAS
A3.17/01, Ref. A58	Schwake J.D.	2001	Storage Stability and Package Corrosion Characteristics of Spinosad Technical: One Year Study, Dow AgroSciences LLC, Formulations Science and Technology Laboratory, Indianapolis, Indiana, USA	Y	Dow (Ref. A58)

	Evaluation by Competent Authorities	
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted	
	EVALUATION BY RAPPORTEUR MEMBER STATE	
Date	27 Nov 2006	
Materials and methods	Table copied from 91/414/EC monograph Hidden texts in the table were made visible via Format-Font-deselect hidden	
Conclusion	-	
Reliability	-	
Acceptability	-	
Remarks	-	
	COMMENTS FROM...	
Date	*Give date of comments submitted*	
Results and discussion	*Discuss additional relevant discrepancies referring to the (sub) heading numbers and to applicant's summary and conclusion.* *Discuss if deviating from view of rapporteur member state*	
Conclusion	*Discuss if deviating from view of rapporteur member state*	
Reliability	*Discuss if deviating from view of rapporteur member state*	
Acceptability	*Discuss if deviating from view of rapporteur member state*	
Remarks		

	Evaluation by Competent Authorities	
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted	
	EVALUATION BY RAPPORTEUR MEMBER STATE	
Date	February 27th, 2009	
Materials and methods	3.1.2, boiling point. Measurements should be continued up to 360 °C. No boiling point was reached prior to decomposition, however. Available data is considered sufficient.	
Conclusion	No boiling point was reached prior to decomposition.	
Reliability	Not applicable	
Acceptability	Acceptable	
Remarks	None	
	COMMENTS FROM...	
Date	*Give date of comments submitted*	
Results and discussion	*Discuss additional relevant discrepancies referring to the (sub) heading numbers and to applicant's summary and conclusion.* *Discuss if deviating from view of rapporteur member state*	

Conclusion	*Discuss if deviating from view of rapporteur member state*
Reliability	*Discuss if deviating from view of rapporteur member state*
Acceptability	*Discuss if deviating from view of rapporteur member state*
Remarks	
	Evaluation by Competent Authorities
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	27 November 2006
Materials and methods	Section IIIA, several sections: IIIA3.1.1 (melting point), IIIA3.4 (UV/VIS, IR, NMR, MS spectra), IIIA3.5 (solubility in water including effect of pH), IIIA3.6 (dissociation constant), IIIA3.7 (solubility in organic solvents), IIIA3.9 (partition coefficient), IIIA3.13 (surface tension). For several physical-chemical properties, data are available for spinosyn A and spinosyn D, but not for spinosad. Although for some of the physical chemical properties (e.g. melting point and solubility in water) values for spinosad (i.e. active substance) will differ from the individual compounds spinosyn A and spinosyn D, these data will give little additional information. Therefore physical-chemical properties for the individual compounds spinosyn A and spinosyn D are considered sufficient. Vapour pressure and Henry"s Law Constant are not available for spinosad, but these are not required because spinosad is a solid substance with an expected vapour pressure < 10^{-5} Pa at ambient temperature.
Conclusion	Several physical chemical properties for the active substance are not available, but data from the individual compounds spinosyn A and D are considered sufficient.
Reliability	-
Acceptability	acceptable
Remarks	
	COMMENTS FROM...
Date	Give date of comments submitted
Results and discussion	Discuss additional relevant discrepancies referring to the (sub) heading numbers and to applicant's summary and conclusion. Discuss if deviating from view of rapporteur member state
Conclusion	Discuss if deviating from view of rapporteur member state
Reliability	Discuss if deviating from view of rapporteur member state
Acceptability	Discuss if deviating from view of rapporteur member state
Remarks	

	Evaluation by Competent Authorities
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted
	EVALUATION BY RAPPORTEUR MEMBER STATE

Date	27 Nov 2006
Materials and methods	Section A3.7 Solubility in organic solvents The effect of temperature on solubility was not verified. Because the biocidal product (GF-739) is a granular solid and does not contain any organic solvents, the effect of temperature on solubility is not relevant in this case.
Conclusion	no comments
Reliability	-
Acceptability	acceptable
Remarks	-
	COMMENTS FROM...
Date	*Give date of comments submitted*
Results and discussion	*Discuss additional relevant discrepancies referring to the (sub) heading numbers and to applicant's summary and conclusion.* *Discuss if deviating from view of rapporteur member state*
Conclusion	*Discuss if deviating from view of rapporteur member state*
Reliability	*Discuss if deviating from view of rapporteur member state*
Acceptability	*Discuss if deviating from view of rapporteur member state*
Remarks	

	Evaluation by Competent Authorities	
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted	
	EVALUATION BY RAPPORTEUR MEMBER STATE	
Date	25 July 2007	
Materials and methods	Section A3.9 Partition coefficient Spinosyn A is considered surface active (surface tension 41.5 mN/m). The shake flask method is not applicable to surface active substances. The calculated log Kow value (EPIsuite version 3.12) is 5.61 for spinosyn A. The measured value for spinosyn A is 3.91. The measured values for the log Kow can be accepted because the solubility in water is so low that this will have no influence on the log Kow measurement.	
Conclusion	no comments	
Reliability	-	
Acceptability	acceptable	
Remarks	-	
	COMMENTS FROM...	
Date	*Give date of comments submitted*	
Results and discussion	*Discuss additional relevant discrepancies referring to the (sub) heading numbers and to applicant's summary and conclusion.* *Discuss if deviating from view of rapporteur member state*	

Conclusion	*Discuss if deviating from view of rapporteur member state*
Reliability	*Discuss if deviating from view of rapporteur member state*
Acceptability	*Discuss if deviating from view of rapporteur member state*
Remarks	

	Evaluation by Competent Authorities	
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted	
	EVALUATION BY RAPPORTEUR MEMBER STATE	
Date	27 Nov 2006	
Materials and methods	Section A3.10 (equivalent to B2.1.3 in the 91/414/EC monograph) Thermal stability, identity of relevant breakdown products Reference is made to the study of the determination of the decomposition temperature. The identity of relevant breakdown products is not determined in this study. A statement or study is necessary. Dow AgroSciences Response March 2006: *"The study (DAS Ref. A18) entitled "Thermogravimetric Analysis of Spinosad and Evolved Gas Analysis by Gas Chromatography/Mass Spectrometry" DECO GL-AL 96-005553, Froelicher, S.W, Feb 1997 has been submitted. This study provides details of the type of substances evolved from spinosad TGAI after heating to high temperatures > 400 deg C.* *Data from this thermogravimetric analysis indicated a sample weight loss of about 92% from the sample up to a temperature of 400 deg. Thermal degradation products associated with this weight loss tentatively identified by TG/GC/MS included carbon dioxide, methyl formate, acetaldehyde, and various sugar fragments of the spinosad molecule."* NL Response April 2006: RMS is satisfied with the answer from DOW.	
Conclusion	After heating to high temperatures (up to 400 °C) a weight loss of 92% is observed. Thermal degradation products included carbon dioxide, methyl formate, acetaldehyde, and various sugar fragments of the spinosad molecule	
Reliability	-	
Acceptability	acceptable	
Remarks	-	
	COMMENTS FROM...	
Date	*Give date of comments submitted*	
Results and discussion	*Discuss additional relevant discrepancies referring to the (sub) heading numbers and to applicant's summary and conclusion.* *Discuss if deviating from view of rapporteur member state*	
Conclusion	*Discuss if deviating from view of rapporteur member state*	
Reliability	*Discuss if deviating from view of rapporteur member state*	
Acceptability	*Discuss if deviating from view of rapporteur member state*	
Remarks		

	Evaluation by Competent Authorities	
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted	
	EVALUATION BY RAPPORTEUR MEMBER STATE	
Date	27 Nov 2006	
Materials and methods	Section A3.11 (equivalent to B2.1.20 in the 91/414/EC monograph) Flammability including auto-flammability and identity of combustion products Reference is made to the study of the determination of the flammability and autoflammability (according to EEC method A10 and A16) The identity of combustion products is not determined in this study. A statement or study is necessary. Dow AgroSciences Response March 2006: "*The flammability test on spinosad (EEC A10) indicated that the material was not flammable. The autoflammability test (EEC A16) on spinosad indicated that the material was not autoflammable and did not self-ignite even up to the maximum temperature of 400°C. We therefore submit that the identity of combustion products from these 2 tests is not relevant or appropriate.*" NL Response April 2006: RMS is satisfied with the answer from DOW.	
Conclusion	The active substance is not flammable, not autoflammable and does not self-ignite up to a maximum temperature of 400 °C. No combustion products are formed.	
Reliability	-	
Acceptability	acceptable	
Remarks	-	
	COMMENTS FROM...	
Date	*Give date of comments submitted*	
Results and discussion	*Discuss additional relevant discrepancies referring to the (sub) heading numbers and to applicant's summary and conclusion.* *Discuss if deviating from view of rapporteur member state*	
Conclusion	*Discuss if deviating from view of rapporteur member state*	
Reliability	*Discuss if deviating from view of rapporteur member state*	
Acceptability	*Discuss if deviating from view of rapporteur member state*	
Remarks		

	Evaluation by Competent Authorities	
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted	
	EVALUATION BY RAPPORTEUR MEMBER STATE	
Date	27 Nov 2006	
Materials and methods	Additional data for A3.1.3 (density); A3.3.1 (physical state); A3.3.2 (colour), A3.3.3 (odour), Ref. A55 The reference stated is incorrect: Karyn, Huntley and Lyn Edgar, 2000, should be changed to: Madsen S, Huntley K, and Edgar L, 2000 The density, physical state, colour and odour was investigated for 3 compounds: spinosyn A (purity 90.9% w/w, batch no TSN 101599), spinosyn D (purity 91.8% w/w, batch no TSN 101600) and spinosad technical (purity 88% total of spinosyn A and D, batch no AGR 293707). The density of spinosyn A, spinosyn D and spinosad technical were determined at 20.5 ± 0.2 °C, 20.4 ± 0.3 °C, and 20.2 ± 0.2 °C, respectively. Relative densities D^{20}_4 calculated against the density of 1.000 g/cm^3 for water at 4 °C, are therefore 1.1244 for spinosyn A, 1.1686 for spinosyn D, and 1.1866 for spinosyn technical. The physical state of spinosyn A, spinosyn D and spinosad technical was determined to be solid at ambient temperature (23.2 °C). The color was investigated using the Munsell Color System at 21.8-23.5 °C. The colour for spinosyn D and spinosad technical was summarized by the applicant. The colour of spinosyn A was determined to have a value of 9, hue of 2.5 Y and a chroma of 3 at 21.8 °C.	
Conclusion	The relative density D^{20}_4 is 1.1244 for spinosyn A, 1.1686 for spinosyn D, and 1.1866 for spinosad technical. The physical state of spinosyn A, spinosyn D and spinosad technical is solid at ambient temperature. The colour of spinosyn A is characterised by a value of 9, hue of 2.5 Y and a chroma of 3 in the Munsell Color System.The colour for spinosyn D is characterised by a value of 9, hue of 5 Y, and a chroma of 1. The colour of spinosad technical is characterised by value of N 9.25, and a percent reflectance of 84.2%. The odour of spinosyn A is fish-like, wax-like, and paint like. The odour of spinosyn D is paint-like, bitter, and aspirin-like. The odour of spinosad technical is chalk-like and dusty.	
Reliability	1	
Acceptability	acceptable	
Remarks		
	COMMENTS FROM...	
Date	*Give date of comments submitted*	
Results and discussion	*Discuss additional relevant discrepancies referring to the (sub) heading numbers and to applicant's summary and conclusion.* *Discuss if deviating from view of rapporteur member state*	
Conclusion	*Discuss if deviating from view of rapporteur member state*	
Reliability	*Discuss if deviating from view of rapporteur member state*	

Acceptability	*Discuss if deviating from view of rapporteur member state*
Remarks	

	Evaluation by Competent Authorities	
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted	
	EVALUATION BY RAPPORTEUR MEMBER STATE	
Date	27 Nov 2006; 11 January 2008	
Materials and methods	Additional data for A3.17 Ref. A58 Initial and post storage active ingredient analysis was carried out by HPLC-UV method DECO ML-AL 94-230319. A description and validation report for this method was provided. Linearity, specificity, repeatability and accuracy were within acceptable limits (sample is dissolved in methanol and analysed by HPLC-UV at 250 nm for both Spinosyn A and D). Samples were stored in flexible polypropylene bags at ambient temperatures (-11 to +32 °C) and an average relative humidity of 58.9% (range 5.6%-91.2%). .	
Conclusion	no comments	
Reliability	1	
Acceptability	Acceptable	
Remarks	None.	
	COMMENTS FROM...	
Date	*Give date of comments submitted*	
Results and discussion	*Discuss additional relevant discrepancies referring to the (sub) heading numbers and to applicant's summary and conclusion.* *Discuss if deviating from view of rapporteur member state*	
Conclusion	*Discuss if deviating from view of rapporteur member state*	
Reliability	*Discuss if deviating from view of rapporteur member state*	
Acceptability	*Discuss if deviating from view of rapporteur member state*	
Remarks		

Section A4 (4.1)	Analytical Methods for Detection and Identification	
Annex Point IIA, IV.4.1	4.1 for the determination of pure active substance	

As described in the "Guidance Document on How to utilize PPP dossiers/monograph" of 21 November 2003 the evaluation of the CA from the PPP monograph (Vol. 3 / Annex B) is used.

The methods have been evaluated in the 91/414/EC Draft Assessment Report (Monograph) on Spinosad of February 2001 in section Spinosadvol3B5 – Annex B – PCP and Methods Section.

The full 91/414/EEC DAR and its addenda are enclosed in the "other documentation" section as described Document I.1 – Application form, point 6.9.

Below is an unchanged copy of the relevant parts of the Spinosad 91/414/EC Draft Assessment Report (DAR) written by the CA (CTB) and released in February 2001. No further information was given in the addenda of June 2002 and May 2005. **DAR numbering has been kept the way it is in the DAR for ease of tracking.**

B.5.1.1 Technical active substance

The validated procedure is a reverse-phase liquid chromatographic method (HPLC) using a C-18 HPLC column. Spinosyn A and Spinosyn D are separated from the other components in the technical material and detected using ultraviolet detection at 250 nm.

The technical sample was dissolved in and diluted with methanol. Chromatography was carried out on a C 18 column with a mobile phase consisting of acetonitrile/methanol and a 2 % ammonium acetate solution (44:44:12 v/v/v). The components were detected by UV at 250 nm.

Validation:

Linearity:

Factor A: linear from 9.72 to 145.8 µg/ml and Factor D: linear from 0.8 to 30 µg/ml

Accuracy: factor A recovery 99.7 % and Factor D 94.6 % in synthetic samples;

Repeatability: RSD = 0.54 % for factor A and RSD= 0.78 % for factor D.

Specifity: based on HPLC-UV

Interference: none detected from chromatogram.

Reference: Frawley, 1994b

An earlier method, used for the characterisation of some of the earlier materials, also uses an HPLC procedure with UV detection. Good linearity over the range 0.004 to 0.560 µg/mL was shown. And a repeatability of <0.63 % for the sum

Reference: Handy, 1991

B.5.1.2 Determination of the impurities in technical material

See confidential section

B.5.5 EVALUATION AND ASSESSMENT

Analytical methods for formulation analysis

Validated methods of analysis are available for the determination of spinosyn A and spinosyn D in technical material and in formulations.

Validated methods are available for the determination of impurities in technical spinosad.

B.5.6 References relied on

91/414 Annex point / reference no. No.	Author(s)	Year	Title Company Report No. Source (where different from company) GLP or GEP status Published or not	Data protection claimed Y/N	Owner
IIA 4.1.1	Frawley, N. N	1994a	Validation of a Method for the Assay of XDE-105 Technical Grade of Active Ingredient by Liquid Chromatography Report no.: 94-230319 GLP Study Unpublished	Y	DAS (O05)
IIA 4.1.1	Handy, P. R.	1991	Determination of LY 232105 in Technical Material Report no.: AM-AA-CA-J422-AA-755 GLP Study Unpublished	Y	DAS (O09)
IIA 4.1.2	Frawley, N. N	1994b	Validation of a Method for the Analysis of Impurities in XDE-105 Technical Grade of Active Ingredient by Liquid Chromatography Report no.: 94-230424 GLP Study Unpublished	Y	DAS (O01)

	Evaluation by Competent Authorities	
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted	
	EVALUATION BY RAPPORTEUR MEMBER STATE	
Date	27 November 2006	
Materials and methods	No comments	
Conclusion	-	
Reliability	-	
Acceptability	acceptable	
Remarks	-	
	COMMENTS FROM...	
Date	Give date of comments submitted	
Results and discussion	Discuss additional relevant discrepancies referring to the (sub) heading numbers and to applicant's summary and conclusion. Discuss if deviating from view of rapporteur member state	
Conclusion	Discuss if deviating from view of rapporteur member state	
Reliability	Discuss if deviating from view of rapporteur member state	
Acceptability	Discuss if deviating from view of rapporteur member state	
Remarks		

Section A4.2 Annex Point IIA, IIA-IV.4.2	Analytical Methods for Detection and Identification 4.2 **Analytical methods on: (a) soil;** (b) air; (c) water; (d) animal and human body fluids and tissues	

As described in the "Guidance Document on How to utilize PPP dossiers/monograph" of 21 November 2003 the evaluation of the CA from the PPP monograph (Vol. 3 / Annex B) is used.

The methods have been evaluated in the 91/414/EC Draft Assessment Report (DAR) on Spinosad of February 2001 in section Spinosadvol3B5 – Annex B – PCP and Methods Section.

The full 91/414/EEC DAR and its addenda are enclosed in the "other documentation" section as described Document I.1 – Application form, point 6.9.

Below is an unchanged copy of the relevant parts of the Spinosad Draft Assessment Report written by the CA (CTB) and released in February 2001 and the 2nd Addendum to the Draft Assessment Report released in May 2005. No further information was given in the 1st addendum of June 2002. Numbering as in the Draft Assessment Report and the Addendum remains unchanged for ease of back tracking.

Taken from the 91/414/EEC Draft Assessment Report of February 2001

B.5.3.1 Residues in soil (Annex IIA 4.2.2)

a) OR19, OR30

Description

Residues of spinosyn A, D, B and N-demethylated spinosyn D are extracted from soil by ultrasonication and shaking with two volumes of a methanol/5% NaCl/1N NaOH (65:27:8 v/v) solution. The two extraction solutions are combined and made up to a known volume with the extraction solution. An aliquot of the extraction solution is purified by partitioning with hexane following the addition 0.16 N hydrochloric acid containing 5% sodium chloride/ (w/v). The hexane layer is discarded. The aqueous layer is made basic by the addition of 1M sodium hydroxide and the spinosyns partitioned into hexane. The hexane is dried with sodium sulphate and evaporated to dryness. The residue is reconstituted in hexane prior to further clean up using a silica SPE cartridge. The silica SPE is washed with hexane, dichloromethane, and acetonitrile before eluting the spinosyns with a methanol/dichloromethane solution (25:75 v/v). The eluate is evaporated to dryness and reconstituted in the HPLC mobile phase where all four spinosyns are determined simultaneously by reversed phase HPLC with UV detection at 250 nm. Quantitative confirmation of the results can be achieved by replacing the C18 analytical column with a mixed phase cation exchange/C18 column and re-injecting the samples. The residues are considered to be confirmed, if the retention times of the analytes matched those of the standards on both columns, and if the confirmatory column gave results that were within ±20% of the results obtained on the primary column.

The method described here, was independently validated at ABC Laboratories, Missouri. During the validation exercise, two minor modifications to the original procedure were introduced. The first modification involved a change in the composition of the HPLC mobile phase to improve separation from co-extracted interferences. The second modification involved the use of hand packed silica gel glass columns to reduce interferences resulting from the silica SPE columns recommended in the method.

Results

The following recovery values (mean ± ó) resulted from fortified samples (n=35) over six concentrations ranging from 0.01 to 1.0 µg/g for spinosyn A, D, B and N-demethylated D: 82±5%, 83±6%, 78±6% and 76±6%. The relative standard deviation (RSD) ranged from 2% to 11% for all four analytes at all fortification levels. The average correlation coefficient (r^2) for the least squares regression equations describing the detector response as a function of the standard calibration curve concentration was 0.9998-0.9999 for all four analytes. The limit of detection was 0.003 µg/g, and the limit of quantification was 0.01 µg/g.

Independent validation of the method showed recoveries of 82±8%, 78±5%, 86±5%, and 77±8% from samples (n=4) fortified at 0.01 or 0.05 µg/g spinosyn A, B, D, and N-demethylated D respectively.

B.5.5 EVALUATION AND ASSESSMENT

Analytical methods (residue) soil, water, air

Where information has been submitted, it fulfils the following criteria:

adequate limit of determination (soil: 0.05 mg/kg, water: 0.1 µg/L);

mean recovery 70-110%;

relative standard deviation of recovery rates <20%;

interfering blanks lower than 30% of the limit of determination;

readily available equipment and reagents used.

In Table B.5.5-1 the method descriptions and validation data are summarised.

Table B.5.5-1 Summary of method description and validation

Substrate	Procedure	Analyte	LOQ	Recovery fortification level	Recoveries range (mean) [%]	Repeatability RSD (n) [%]	Linearity demonstrated	Reference
soil	Extraction with methanol/5% sodium chloride/1N sodium hydroxide (65:27:8 v/v), clean up by silica SPE cartridge, analysis by HPLC-UV (250 nm)	spinosyn A spinosyn D spinosyn B N-demethylated spinosyn D	0.01 mg/kg	0.010-1.0 mg/kg	71-89 (82) 71-95 (83) 64-87 (78) 61-85 (76)	6.3 (n=35) 7.8 (n=35) 7.1 (n=35) 7.7 (n=35	yes	OR19
soil		spinosyn A spinosyn D spinosyn B N-demethylated spinosyn D	0.01 mg/kg	0.01-0.05 mg/kg	72-89 (82) 82-93 (86) 72-84 (78) 66-84 (77)	9.4 (n=4) 6.1 (n=4) 6.5 (n=4) 9.9 (n=4)		OR30

B.5.6 B5.6 References relied on

91/414 Annex point / reference no. No.	Author(s)	Year	Title Company Report No. Source (where different from company) GLP or GEP status Published or not	Data protection claimed Y/N	Owner
IIA 4.2.2	West, S.D.	1995	Determination of XDE-105 and Metabolites in Soil and Sediment by High Performance Liquid Chromatography with Ultraviolet Detection. DowElanco, Report No. GRM 94.20 Ref. OR19 GLP Study Unpublished	Y	DAS (OR19)
IIA 4.2.2	Lochhaas, C.	1995	Independent Laboratory Evaluation of Method gRM 94.20 - Determination of XDE-105 and Metabolites in Soil and Sediment by High Performance Liquid Chromatography with Ultraviolet Detection. ABC Laboratories Inc., Report No. gH-C 3212 Ref. OR30 GLP Study Unpublished	Y	DAS (OR30)

Taken from the 91/414/EEC 2nd Addenda to the Draft Assessment Report of May 2005

B.5.3 Analytical methods (residue) soil, water, air (Annex IIA 4.2.2 to 4.2.4; Annex IIIA 5.2)

B.5.3.1 Residues in soil (Annex IIA 4.2.2)

Study OR94

Reference/notifier	:	Hastings, M.J.	GLP statement	:	yes
Type of study	:	validation analysis method soil	Guideline	:	not applicable
Year of execution	:	2003	Acceptability	:	partly acceptable
Test substance	:	spinosyn A, TSN102499, purity 91.2% spinosyn D, TSN101600, purity 94,0% spinosyn B, TSN102302, purity 94,0% N-demethyl spinosyn D, TSN100724, purity 97,4%			

Substrate	Analyte	LOQ [µg/g]	Recovery fortification level [µg/g]	Recoveries: range (mean) [%]	Repeatability RSD (n) [%]	Linearity of response (r^2)
sandy loam soil	spinosyn A	0.25	0.25	86-96 (91.0)	3.9 (6)	
			1.0	88-100 (93.8)	4.2 (6)	
	spinosyn D	0.25	0.25	84-96 (90.2)	5.2 (6)	
			1.0	88-97 (93.2)	3.8 (6)	
	spinosyn B	0.25	0.25	85-94 (89.2)	3.7 (6)	
			1.0	88-95 (92.3)	3.7 (6)	
	N-demethyl spinosyn D	0.25	0.25	86-95 (89.3)	4.1 (6)	
			1.0	87-95 (92.2)	3.2 (6)	

Description

Method validation.
Soil and sediment were applied with a stock of each of the test substances in methanol/acetonitrile (1:1). Fortification levels 0.005, 0.05, 0.25, and 1.00 µg/L. Representative samples of soil and sediment matrix were fortified at 0.001 µg/g to demonstrate the method LOD.

ANALYSIS METHOD
GRM 03.19. Residues of spinosad and its metabolites are extracted from soil samples by shaking with a solution of methanol/5% NaCl/ 1N NaOH. An aliquot of the extraction solvent is diluted with 10% NaCl, and spinosad and its metabolites are partitioned into methyl *tert*-butyl ether. After evaporation to dryness, the residues are reconstituted in a solution of acetonitrile/methanol/water containing 0.1% ammonium acetate. The final solution is analysed by LC/MS/MS.

Results

The method was validated over the concentration range of 0.25-1.0 µg/g with a LOQ of 0.005 µg/g.

Remarks by RMS

Soil types tested were: loam, clay loam, silt, sandy loam, silt loam, silty clay loam, sandy clay loam and loamy sand. Sediment types tested were: sandy clay loam, loamy sand, sandy loam, and loam. According to the Guidance document on residue analytical methods (SANCO/825/00 rev. 7, 17-03-2004), the sample set should consist of 5 samples per tested concentration per substrate. This condition was only met for the sandy loam soil at concentrations of 0.25 and 1.00 µg/g. Therefore only the results of this part of the study are used for the risk assessment.

B.5.6 References relied on

91/414/EEC Annex point/ reference no.	Author(s)	Year	Title Company, report no. Source (where different from company) GLP or GEP status (where relevant) Published or not	Data Protection Claimed Y/N	Owner
IIA 4.2.1	Rawle NW	2003	Independent laboratory validation of analytical methods for the determination of spinosad residues in crops with a high aqueous content, crops with an oil content, dry and acidic crops. Dow AgroSciences, PTR number 153 553 29-5008-1 CEM Analytical Services, Berkshire, UK, report no CEMR-1373 GLP Not published	Y	DAS (OR 91)
IIA, 4.2.2	Hastings, M.J.	2003	Validation of Dow AgroSciences Method GRM 0.319 – Determination of spinosad and its metabolites in soil and sediment by Liquid Chromatography with Tandem Mass Spectrometry.	Y	DAS (OR 94)

Section A4 (4.2) **Annex Point IIA, IIA-IV.4.2**	**Analytical Methods for Detection and Identification** Specify where appropriate, e.g. isomer of a.s., metabolite of a.s., impurity of a.s., matrix 4.1 for the determination of pure active substance 4.2 **Analytical methods on: (a) soil;** (b) air; (c) water; (d) animal and human body fluids and tissues	
	4 REFERENCES (98/8 REF. A4.2/01, OR94)	**Official use only**
4.1 Reference	Hastings, M. J., 2003, Validation of Dow AgroSciences Method GRM 03.19 - Determination of Spinosad and its Metabolites in Soil and Sediment by Liquid Chromatography with Tandem Mass Spectrometry, Reg. Labs—Indianapolis, Indiana, USA, (08-July-2003) **(Ref. A4.2/01, OR94)**	
4.2 Data protection	Yes	
4.2.1 Data owner	Dow AgroSciences LLC	
4.2.2 Companies with letter of access	None	
4.2.3 Criteria for data protection	Data on new active substance for first entry to Annex I authorisation	
	5 GUIDELINES AND QUALITY ASSURANCE	
5.1 Guideline study	Yes	
5.2 GLP	Yes	
5.3 Deviations	No	
	6 MATERIALS AND METHODS	
6.1 Preliminary treatment		
6.1.1 Enrichment	Residues of spinosad and its metabolites are extracted from a 5-g soil sample by shaking for 30 minutes with a methanol/5% sodium chloride/1N sodium hydroxide (65:27:8) solution. The sample is centrifuged and the extraction solution is decanted into a graduated mixing cylinder. The extraction process is repeated with a second aliquot of the extraction solution. The extracts are combined and made up to a known volume with the extraction solution.	
6.1.2 Cleanup	An aliquot of the extraction solvent is diluted with 10% sodium chloride, and spinosad and its metabolites are partitioned into methyl tert-butyl ether (MTBE). The MTBE is evaporated to dryness and the residues are reconstituted in an acetonitrile/methanol/water (4:4:2) solution containing 0.1% ammonium acetate.	
6.2 Detection		
6.2.1 Separation method	The final solution is analyzed by gradient high performance liquid chromatography using a YMC ODS-AM column (5-µm, 150 x 4.6 mm i.d.) with a acetonitrile:methanol:water:acetic acid mobile phase.	
6.2.2 Detector	Detection of spinosad and it metabolites is performed with positive-ion atmospheric pressure chemical ionization (APCI) tandem mass	

Section A4 (4.2) **Annex Point IIA, IIA-IV.4.2**	**Analytical Methods for Detection and Identification** Specify where appropriate, e.g. isomer of a.s., metabolite of a.s., impurity of a.s., matrix 4.1 for the determination of pure active substance 4.2 **Analytical methods on: (a) soil;** (b) air; (c) water; (d) animal and human body fluids and tissues	
	spectrometry (MS/MS) monitoring analyte specific precursor-ion/product-ion transitions (fragments) of the spinosyns as follows: Spinosyn A *m/z* Q1/Q3 732.5/142.1 Spinosyn D *m/z* Q1/Q3 746.5/142.1 Spinosyn B *m/z* Q1/Q3 718.5/128.1 *N*-demethyl spinosyn D *m/z* Q1/Q3 732.5/128.1	
6.2.3 Standard(s)	Quantification of residues of spinosad and its metabolites is performed using an external calibration standard technique.	
6.2.4 Interfering substance(s)	During the validation study, 20 different soils and 2 different sediment samples were analysed. No interferences from co-extracted species were observed.	
6.3 Linearity		
6.3.1 Calibration range	0.0001 – 0.05 µg/mL (0.1 – 50 ng/mL). Equivalent to 0.001 – 0.50 µg/g spinosyns A and D and their metabolites spinosyn B and *N*-demethyl spinosyn D in soil.	
6.3.2 Number of measurements	8 standards were injected throughout the analytical run.	
6.3.3 Linearity	The calibration curves from 5 analytical runs yielded correlation coefficients (r) of at least 0.9997.	

Section A4 (4.2)

Annex Point IIA, IIA-IV.4.2

Analytical Methods for Detection and Identification

Specify where appropriate, e.g. isomer of a.s., metabolite of a.s., impurity of a.s., matrix

4.1 for the determination of pure active substance

4.2 **Analytical methods on: (a) soil;** (b) air; (c) water; (d) animal and human body fluids and tissues

6.4 Specifity: interfering substances

HPLC/MS/MS affords a highly specific method for both quantification and confirmation of residue identity by retention time matching in conjunction with monitoring the specific MS/MS ion transitions of spinosad and its metabolites.

6.5 Recovery rates at different levels

Analyte	Fortification Level (µg/g)	Average Recovery (%)	Recovery Range (%)	n
Spinosyn A	0 005	96	81 - 111	14
	0 05	88	76 - 93	14
	0 25	92	86 - 98	14
	1 00	91	79 - 100	14
	0 005-1 00	92	76 - 111	56
Spinosyn D	0 005	94	86 - 107	14
	0 05	88	74 - 96	14
	0 25	92	84 - 101	14
	1 00	90	78 - 97	14
	0 005-1 00	91	74 - 107	56
Spinosyn B	0 005	88	80 - 101	14
	0 05	86	76 - 93	14
	0 25	90	83 - 97	14
	1 00	89	80 - 98	14
	0 005-1 00	88	76 - 101	56
N-demethyl Spinosyn D	0 005	88	77 - 101	14
	0 05	86	78 - 92	14
	0 25	90	83 - 95	14
	1 00	89	77 - 95	14
	0 005-1 00	88	77 - 101	56

Section A4 (4.2) **Annex Point IIA, IIA-IV.4.2**	**Analytical Methods for Detection and Identification** Specify where appropriate, e.g. isomer of a.s., metabolite of a.s., impurity of a.s., matrix 4.1 for the determination of pure active substance 4.2 **Analytical methods on: (a) soil;** (b) air; (c) water; (d) animal and human body fluids and tissues	

6.5.1 Relative standard deviation

Analyte	Fortification Level (µg/g)	SD (%)	RSD (%)	n
Spinosyn A	0 005	7 6	7 9	14
	0 05	5 0	5 6	14
	0 25	3 6	3 6	14
	1 00	5 9	6 5	14
	0 005-1 00	6 2	6 8	56
Spinosyn D	0 005	6 2	6 6	14
	0 05	5 8	6 5	14
	0 25	4 9	5 3	14
	1 00	5 7	6 3	14
	0 005-1 00	5 9	6 5	56
Spinosyn B	0 005	6 5	7 4	14
	0 05	4 6	5 4	14
	0 25	4 0	4 4	14
	1 00	5 9	6 6	14
	0 005-1 00	5 5	6 2	56
N-demethyl Spinosyn D	0 005	8 2	9 3	14
	0 05	4 5	5 2	14
	0 25	3 9	4 3	14
	1 00	5 7	6 4	14
	0 005-1 00	5 8	6 6	56

Section A4 (4.2) **Annex Point IIA, IIA-IV.4.2**	**Analytical Methods for Detection and Identification** Specify where appropriate, e.g. isomer of a.s., metabolite of a.s., impurity of a.s., matrix 4.1 for the determination of pure active substance 4.2 **Analytical methods on: (a) soil;** (b) air; (c) water; (d) animal and human body fluids and tissues	
6.6 Limit of determination	Following established guidelines (1), the limits of quantitation (LOQ) and detection (LOD) were calculated for spinosad and its metabolites using the standard deviation for the 0.005-µg/g (LOQ) recovery results. The LOQ was calculated as ten times the standard deviation (10s), and the LOD was calculated as three times the standard deviation (3s) of the analysis of a minimum of 14 samples. The results are summarized below. The calculated LOQ supports the validated method LOQ of 0.005 µg/g. The calculated LOD's were in the range of 0.00093 – 0.00123 µg/g which supports a method LOD of 0.001 µg/g. *(1) Keith, L. H.; Crummett, W.; Deegan, J., Jr.; Libby, R. A.; Taylor, J. K.; Wentler, G. Anal. Chem.* ***1983****, 55, 2210-2218.*	
6.7 Precision		
6.7.1 Repeatability	No specific repeatability data was generated. However, the data presented in section 3.5, 3.5.1, and 3.6 is a composite of five analytical validation batches generated over a period of approximately three weeks.	
6.7.2 Independent laboratory validation	No independent validation was conducted on method GRM 03.19.	

Analyte	Average Recovery (µg/g)	Standard Deviation (*s*)	Limit of Detection (3*s*)	Limit of Quantitation (10*s*)
Spinosyn A	0.00479	0.00038	0.00115	0.00383
Spinosyn D	0.00471	0.00031	0.00093	0.00309
Spinosyn B	0.00442	0.00033	0.00098	0.00327
N-demethyl Spinosyn D	0.00438	0.00041	0.00123	0.00408

Section A4 (4.2) **Annex Point IIA, IIA-IV.4.2**	**Analytical Methods for Detection and Identification** Specify where appropriate, e.g. isomer of a.s., metabolite of a.s., impurity of a.s., matrix 4.1 for the determination of pure active substance 4.2 **Analytical methods on: (a) soil;** (b) air; (c) water; (d) animal and human body fluids and tissues	
	7 **APPLICANT'S SUMMARY AND CONCLUSION**	
7.1 Materials and methods	Dow AgroSciences method GRM 03.19 is applicable for the quantitative determination of residues of spinosad and its metabolites in soil and sediment. The method was validated over the concentration range of 0.005-1.0 µg/g. The validated limit of quantification for the method is 0.005 µg/g. Residues of spinosad and its metabolites are extracted from soil samples by shaking with a methanol/5% sodium chloride/1N sodium hydroxide solution (65:27:8). An aliquot of the extraction solvent is diluted with 10% sodium chloride and spinosad and its metabolites are partitioned into methyl *tert*-butyl ether (MTBE). The MTBE is evaporated to dryness and the residues are reconstituted in an acetonitrile/methanol/water (4:4:2) solution containing 0.1% ammonium acetate. The final solution is analyzed by liquid chromatography with positive ion atmospheric pressure chemical ionization (APCI) tandem mass spectrometry (LC/MS/MS). A calibration curve resulting from the injection of eight standards demonstrated linearity with a correlation coefficient of at least 0.9997. LC/MS/MS affords a highly specific method for quantification and confirmation of spinosad and its metabolites by retention time matching with standards in conjunction with monitoring analyte specific precursor-ion/product-ion transitions.	
7.2 Conclusion	The data summarized below demonstrates the suitability of method GRM 03.19 for the analysis of spinosad and its metabolite residues in soil and sediment.	
7.2.1 Reliability	1	
7.2.2 Deficiencies	No	

Analyte	Fortification Level (µg/g)	Average Recovery (%)	Recovery Range (%)	SD (%)	RSD (%)
Spinosyn A	0.005– 1.0	92	76 – 111	6.2	6.8
Spinosyn D	0.005– 1.0	91	74 – 107	5.9	6.5
Spinosyn B	0.005– 1.0	88	76 – 101	5.5	6.2
N-demethyl spinosyn D	0.005– 1.0	88	77 – 101	5.8	6.6

	Evaluation by Competent Authorities	
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted	

	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	26 July 2007
Materials and methods	See Remarks
Conclusion	See Remarks
Reliability	See Remarks
Acceptability	See Remarks
Remarks	Based on the same studies, the following conclusion was drawn in the second Addendum to the DAR, issued in May/July 2005 and revised in March 2006: Validity of analysis method GRM 03.19 for the determination of spinosyn A and D, and their metabolites spinosyn B and N-demethyl spinosyn D is demonstrated in different soil types. The method was validated with an LOQ of 0.005 mg/kg for each compound.
	COMMENTS FROM...
Date	*Give date of comments submitted*
Results and discussion	*Discuss additional relevant discrepancies referring to the (sub) heading numbers and to applicant's summary and conclusion.* *Discuss if deviating from view of rapporteur member state*
Conclusion	*Discuss if deviating from view of rapporteur member state*
Reliability	*Discuss if deviating from view of rapporteur member state*
Acceptability	*Discuss if deviating from view of rapporteur member state*
Remarks	

Section A4.2 Annex Point IIA, IIA-IV.4.2	Analytical Methods for Detection and Identification 4.2 **Analytical methods on:** (a) soil; **(b) air**; (c) water; (d) animal and human body fluids and tissues	

As described in the "Guidance Document on How to utilize PPP dossiers/monograph" of 21 November 2003 the evaluation of the CA from the PPP monograph (Vol. 3 / Annex B) is used.

The methods have been evaluated in the 91/414/EC Draft Assessment Report (DAR) on Spinosad of February 2001 in section Spinosadvol3B5 – Annex B – PCP and Methods Section.

The full 91/414/EEC DAR and its addenda are enclosed in the "other documentation" section as described Document I.1 – Application form, point 6.9.

Below is an unchanged copy of the relevant parts of the Spinosad Draft Assessment Report (DAR) written by the CA (CTB) and released in February 2001 and the 2nd addendum to the DAR of May 2005. No further information is given in the 1st addendum to the DAR of June 2002. Numbering as in the DAR and the addendum remains unchanged for ease of back tracking.

Taken from the 91/414/EEC Draft Assessment Report of February 2001

B.5.3.3 Residues in air (Annex IIA 4.2.4)

a) OR75

Description

Air is sampled with an OVS tube containing glass fibre filter to collect aerosol and XAD-2 to collect vapour that passes through the filter.For method validation OVS tubes were fortified with 11.6, 141 and 1320 µg Spinosad/tube. The filters were air dried for about 10 minutes. Then 80 % relative hunidity-air was pulled through each tube at 1 L/min for 8 hours. Spinosad was desorbed with acetonitril.

Residues of spinosyn A and spinosyn B were analysed by reversed phase HPLC on a C-18 column, with a mobile phase consisting of 0.01 M ammonium acetate in 90/10 acetoniil/water. UV detection was carried out at 254 nm.

Validation:

At a air-humidity of 80 %

Linearity was demonstrated for 2 to 2000 µg spinosad.

Recovery: 65.8 % for tubes fortified with 11.6 µ, 84.1 % for tubes fortified with 141 µ and 93.1 % for samples fortified at 1320 µg.

Based on a through put of 480 liter air, the calculated LOQ based on the 141 µg addition is 0.294 mg/m^3.

B.5.5 EVALUATION AND ASSESSMENT

Analytical methods (residue) soil, water, air

Where information has been submitted, it fulfils the following criteria:

adequate limit of determination (soil: 0.05 mg/kg, water: 0.1 µg/L);

mean recovery 70-110%;

relative standard deviation of recovery rates <20%;

interfering blanks lower than 30% of the limit of determination;

readily available equipment and reagents used.

In Table B.5.5-1 the method descriptions and validation data are summarised.

Title, level, chapter

Table B.5.5-1 Summary of method description and validation

Substrate	Procedure	Analyte	LOQ	Recovery fortification level	Recoveries range (mean) [%]	Repeatability RSD (n) [%]	Linearity demonstrated	Reference
air	Air is sampled with an OVS tube containing glass fibre filter to collect aerosol and XAD-2 to collect vapour that passes through the filter. Separate sections are extracted and analysed by HPLC.	Spinosyn A and D	0.294 mg/m3	11.6 µg; 141 ug 1320 µg	65.8 % (n=8) 84.1 % (n=8) 93.1 % (n=7)	7.9 1.1 3.9	Y	~~OR76~~ is OR75

B5.6 References relied on

91/414 Annex point / reference no. No.	Author(s)	Year	Title Company Report No. Source (where different from company) GLP or GEP status Published or not	Data protection claimed Y/N	Owner
IIA 4.2.4	Huff, D.W.	1999	Development and Validation of an Industrial Hygiene Air Monitoring Method for Spinosad PROTOCOL. The Dow Chemical Company, Report No. HEH 26008 Ref. OR75 GLP Study Unpublished	Y	DAS

Taken from the 2nd addendum to the 91/414/EEC Draft Assessment Report of May 2005

B.5.3.3 Residues in air (Annex IIA 4.2.4)

Study OR92

Reference/notifier	:	Atkinson, S.	GLP statement	:	yes
Type of study	:	analytical method air, validation	Guideline	:	not applicable
Year of execution	:	2002	Acceptability	:	acceptable
Test substance	:	spinosyn A spinosyn D			

Substrate	Analyte	T [C]	RH [%]	LOQ [µg/m^3]	Recovery fortification level [µg/m^3]$^{\#}$	Recoveries: range (mean) [%]$^{\$}$	Repeatability RSD (n) [%]	Linearity of response (r^2)
air	spinosyn A	21.5 22.3 22	44.8 43.2 42.8	0.73	0.73 7.3 73	74-77 (76) 85-89 (88) 84-94 (90)	1.72 (5) 1.91 (5) 4.26 (5)	1.000
air	spinosyn A	35.2 35.0 35.0	79.7 79.6 79.6	0.73	0.73 7.3 73	92-94 (93) 76-85 (81) 92-101 (94)	1.08 (5) 4.00 (5) 4.14 (5)	1.000
air	spinosyn D	21.5 22.3 22	44.8 43.2 42.8	0.73	0.73 7.3 73	75-80 (77) 85-93 (89) 85-96 (91)	2.60 (5) 3.42 (5) 4.73 (5)	1.000
air	spinosyn D	35.2 35.0 35.0	79.7 79.6 79.6	0.73	0.73 7.3 73	92-95 (93) 75-87 (82) 89-93 (91)	1.40 (5) 5.53 (5) 1.55 (5)	1.000

$^{\#}$: based on spinosad
$^{\$}$: recovery expressed as percentages of nominal amounts of spinosad added to the tubes.

Description

Analysis method GRM 02.18
A measured volume of air is drawn through a commercial Tenax two-segment configured adsorption tube. After air sampling, the front and back-up beds are extracted with a solution of methanol, acetonitrile and aqueous ammonium acetate. An aliquot of the extract is analysed by HPLC with +APCI mass spectroscopy detection (LC-MS/MS).
Validation

Extractability

Tubes of Tenax adsorbent are fortified in triplicate to give loadings of 0.259, 2.59 and 25.9 µg spinosad in methanol/acetonitrile 50/50 (v/v) along with unfortified control tubes containing 100 µL of methanol/acetonitrile (50/50) (v/v) only. The loadings are equivalent to air concentrations of 0.73, 7.3 and 73 µg/m^3.
The test solutions were applied with a 100 µL glass syringe to the front portion of the tube packing. After allowing the solvent to evaporate, the tubes were analysed according to the method described below.
Breakthrough
Air with the following characteristics was used: ambient temperature and relative humidity, and at 35 °C and 80% relative humidity. For each set of conditions, 1 control tube and 5 tubes fortified at 0.264 µg, 2,64 µg and 26,4 µg were tested. After the 6-hour period, the tubes were separated into front and back segments and analysed.
Storage stability
The storage stability of spinosad in extracts from Tenax adsorbent tubes was measured after 4 and 7 days of storage at room temperature and at 4 °C. Three fortified tubes were stored at <18 °C, 4 °C and ambient room temperature for 4 and 8 days prior to analysis.

Results

The LOQ was 0.73 µg/m^3 for spinosyn A and spinosyn D, respectively. No significant breakthrough was observed in any of the rear segments. Linearity was reported to be 1.000 for both spinosyn A and D.

Remarks by RMS

Significant loss of the analyte was seen after 8 days when tubes were stored at ambient room temperature. Purity of test substances not reported.

.5.6 References relied on

91/414/EEC Annex point/ reference no.	Author(s)	Year	Title Company, report no. Source (where different from company) GLP or GEP status (where relevant) Published or not	Data Protection Claimed Y/N	Owner
IIA, 4.2.4	Atkinson, S.	2002	Determination of residues of spinosad in air by high performance liquid chromatography with +APCI Mass Spectroscopy detection	Y	DAS (OR 92)

Section A4 (4.2) Annex Point IIA, IIA-IV.4.2	Analytical Methods for Detection and Identification Specify where appropriate, e.g. isomer of a.s., metabolite of a.s., impurity of a.s., matrix 4.1 for the determination of pure active substance 4.2 **Analytical methods on**: (a) soil; **(b) air**; (c) water; (d) animal and human body fluids and tissues	
	8 REFERENCES (REF. A4.2/02, OR92)	**Official use only**
8.1 Reference	Atkinson, S., Determination of Residues of Spinosad in Air by High Performance Liquid Chromatography with +APCI Mass Spectroscopy Detection, CEM Analytical Services Ltd, North Ascot, Berkshire, UK, (Dow AgroSciences LLC Method GRM 02.18) (04-July-2002) **(Ref. A4.2/02, OR92)**	
8.2 Data protection	Yes	
8.2.1 Data owner	Dow AgroSciences LLC	
8.2.2 Companies with letter of access	None	
8.2.3 Criteria for data protection	Data on new active substance for first entry to Annex I authorisation	
	9 GUIDELINES AND QUALITY ASSURANCE	
9.1 Guideline study	Yes	
9.2 GLP	Yes	
9.3 Deviations	No	
	10 MATERIALS AND METHODS	
10.1 Preliminary treatment		
10.1.1 Enrichment	Simulated sampling was conducted at ambient temperature and humidity and again at elevated temperature and humidity (35 °C and ≥ 80% r h.). A measured volume of air is drawn through a commercial Tenax two-segment configured adsorption tube for 6 hours at a flow rate of 1 L/minute. After air sampling, spinosad (spinosyns A and D) is extracted from the tube adsorbent with a methanol, acetonitrile, and aqueous ammonium acetate solution.	
10.1.2 Cleanup	The sample is centrifuged to separate the adsorbent from the extract. An aliquot of the sample extract is transferred to an autosampler vial for analysis.	
10.2 Detection		
10.2.1 Separation method	The final sample extract is analyzed by gradient high performance liquid chromatography using a Phenomenex Prodigy column (5-µm, 100 x 4.6 mm i.d.) with a acetonitrile methanol:water:ammonium acetate mobile phase.	
10.2.2 Detector	Detection of spinosad is performed with positive-ion atmospheric pressure chemical ionization (APCI) tandem mass spectrometry (MS/MS) monitoring analyte specific precursor-ion/product-ion transitions (fragments) of the spinosad as follows:	

Section A4 (4.2) **Annex Point IIA, IIA-IV.4.2**	**Analytical Methods for Detection and Identification** Specify where appropriate, e.g. isomer of a.s., metabolite of a.s., impurity of a.s., matrix 4.1 for the determination of pure active substance 4.2 **Analytical methods on**: (a) soil; **(b) air**; (c) water; (d) animal and human body fluids and tissues	
	Spinosyn A *m/z* Q1/Q3 732.6/142.2 Spinosyn D *m/z* Q1/Q3 746.6/142.2	
10.2.3 Standard(s)	Quantitation of residues of spinosad (spinosyns A and D) is performed using an external calibration standard technique.	
10.2.4 Interfering substance(s)	During the validation study, 5 replicates at each of 3 different concentration levels were analysed. No interferences from the extraction of the unfortified control tubes were observed.	
10.3 Linearity		
10.3.1 Calibration range	0.005 – 0.20 µg/mL (5.0 – 200 ng/mL). An adsorbent tube fortified with spinosad at the validated limit of quantification of the method at 0.264 µg per tube is equivalent to 0.73 µg/m^3 based on a 360-L air volume. (Solutions containing more than 0.2 µg/mL of spinosad were diluted to keep them within the range of the calibration curve.)	
10.3.2 Number of measurements	Six (6) standards of each, spinosyns A and D were injected throughout the analytical run.	
10.3.3 Linearity	The typical calibration curves shown yielded correlation coefficients (r) of 1.0000 for both spinosyns A and D.	
10.4 Specifity: interfering substances	HPLC/MS/MS affords a highly specific method for both quantitation and confirmation of residue identity by retention time matching in conjunction with monitoring the specific MS/MS ion transitions of spinosad (spinosyns A and D).	
10.5 Recovery rates at different levels		

Analyte	Matrix[a]	Fortification Level (µg)	Fortification Level (µg)[b]	Average Recovery (%)	Recovery Range (%)	n
Spinosyn A	Air (ambient)	0 264	0 228	76	74 - 77	5
		2 64	2 28	88	85 - 89	5
		26 4	22 8	90	84 - 94	5
		0 264 – 26 4	0 228 – 22 8	85	77 - 94	15
Spinosyn A	Air (elevated)	0 264	0 228	93	92 - 94	5
		2 64	2 28	81	76 - 85	5
		26 4	22 8	94	92 - 101	5
		0 264 – 26 4	0 228 – 22 8	89	76 - 101	15
Spinosyn D	Air (ambient)	0 264	0 0360	77	75 - 80	5
		2 64	0 360	89	85 - 93	5
		26 4	3 60	91	85 - 96	5
		0 264 – 26 4	0 0360 – 3 60	86	75 - 96	15
Spinosyn D	Air (elevated)	0 264	0 0360	93	92 - 95	5
		2 64	0 360	82	75 - 87	5
		26 4	3 60	91	89 - 93	5
		0 264 – 26 4	0 0360 – 3 60	89	75 - 95	15

[a]Ambient air conditions are approximately 22 °C and 43% relative humidity Elevated air conditions are approximately 35 °C and 80% relative humidity
[b]Fortification based on weight percent of spinosyns A and D – weight percent: 82 3% spinosyn A and 13 0% spinosyn D (overall purity of 95 3%)

Section A4 (4.2)

Annex Point IIA, IIA-IV.4.2

Analytical Methods for Detection and Identification

Specify where appropriate, e.g. isomer of a.s., metabolite of a.s., impurity of a.s., matrix

4.1 for the determination of pure active substance

4.2 **Analytical methods on**: (a) soil; **(b) air**; (c) water; (d) animal and human body fluids and tissues

10.5.1 Relative standard deviation

Analyte	Matrix[a]	Fortification Level (µg)	Fortification Level (µg)[b]	SD (%)	RSD (%)	n
Spinosyn A	Air (ambient)	0.264	0.228	1.30	1.72	5
		2.64	2.28	1.67	1.91	5
		26.4	22.8	3.85	4.26	5
		0.264 – 26.4	0.228 – 22.8	6.96	8.22	15
Spinosyn A	Air (elevated)	0.264	0.228	1.00	1.08	5
		2.64	2.28	3.24	4.00	5
		26.4	22.8	3.91	4.14	5
		0.264 – 26.4	0.228 – 22.8	6.81	7.61	15
Spinosyn D	Air (ambient)	0.264	0.0360	2.00	2.60	5
		2.64	0.360	3.03	3.43	5
		26.4	3.60	4.30	4.73	5
		0.264 – 26.4	0.0360 – 3.60	7.04	8.22	15
Spinosyn D	Air (elevated)	0.264	0.0360	1.30	1.40	5
		2.64	0.360	4.56	5.53	5
		26.4	3.60	1.41	1.55	5
		0.264 – 26.4	0.0360 – 3.60	5.50	6.19	15

[a]Ambient air conditions are approximately 22 C and 43% relative humidity. Elevated air conditions are approximately 35 C and 80% relative humidity.
[b]Fortification based on weight percent of spinosyns A and D – weight percent: 82.3% spinosyn A and 13.0% spinosyn D (overall purity of 95.3%).

10.6 Limit of determination

Following established guidelines (1), the limits of quantitation (LOQ) and detection (LOD) were calculated for spinosad in Tenax adsorbent tubes were calculated using the standard deviation from the 0.264-µg per adsorbent tube extractability results. The LOQ was calculated as ten times the standard deviation (10s), and the LOD was calculated as three times the standard deviation (3s) of the analysis of 3 samples. The results are summarized below.

Analyte	Average Recovery (µg)[a]	Standard Deviation (*s*)	Limit of Detection (3*s*)	Limit of Quantitation (10*s*)
Spinosyn A	0.2147	0.0017	0.0052	0.0174
Spinosyn D	0.0346	0.0005	0.0014	0.0046

[a]Fortification was based on weight percent of spinosyns A and D. Weight percent: 82.3% spinosyn A and 13.0% spinosyn D (overall purity of 95.3%).

The calculated LOQ's for spinosad support the validated method LOQ of 0.264 µg per adsorbent tube. The calculated LOD's for spinosad support a method LOD of 0.0792 µg per adsorbent tube.

(1) Keith, L. H.; Crummett, W.; Deegan, J., Jr.; Libby, R. A.; Taylor, J. K.; Wentler, G. Anal. Chem. ***1983****, 55, 2210-2218.*

10.7 Precision

10.7.1 Repeatability

The data presented in section 3.5 and 3.5.1 is the result of five replicate

Section A4 (4.2) **Annex Point IIA, IIA-IV.4.2**	**Analytical Methods for Detection and Identification** Specify where appropriate, e.g. isomer of a.s., metabolite of a.s., impurity of a.s., matrix 4.1 for the determination of pure active substance 4.2 **Analytical methods on**: (a) soil; **(b) air**; (c) water; (d) animal and human body fluids and tissues	
	sample recoveries generated at three different concentration levels under ambient conditions and again at elevated temperature and humidity. The data presented in section 3.6 is the result of three replicate sample recoveries generated at three different concentration levels primarily for the purpose of showing extraction efficiency.	
10.7.2 Independent laboratory validation	No independent validation was conducted on method GRM 02.18.	
	11 APPLICANT'S SUMMARY AND CONCLUSION	
11.1 Materials and methods	Dow AgroSciences method GRM 02.18 is applicable for the quantitative determination of residues of spinosad (spinosyns A and D) in air. The method was validated over the concentration range of 0.73 µg/m^3 to 73 µg/m^3. The validated limit of quantitation for the method is 0.264 µg per adsorbent tube or 0.73 µg/m^3 based on a 360-L air volume. A measured volume of air is drawn through a commercial Tenax two-segment configured adsorption tube for 6 hours at a flow rate of 1 L/minute. After air sampling, the sorbent is removed from Tenax adsorbent sample tubes. Residues of spinosad are extracted from samples by shaking vigorously for 30 minutes with 10.0 mL of a 95:5 solution (A:B) where A = methanol/acetonitrile 1:1 (v/v) + 0.1% (w/v) and B = deinozed water + 0.1% ammonium acetate. An aliquot of the sample extract is transferred to an autosampler vial for analysis without additional cleanup. Samples that exceed the range of the calibration curve are diluted to keep the concentrations within the range of the curve. The final solution and along with the calibration standards is analyzed by liquid chromatography with positive ion atmospheric pressure chemical ionization (APCI) tandem mass spectrometry (LC/MS/MS). A calibration curve resulting from the injection of six standards demonstrated linearity with a typical correlation coefficient (r) of 1.0000. LC/MS/MS affords a highly specific method for quantitation and confirmation of spinosad by retention time matching with standards in conjunction with monitoring analyte specific precursor-ion/product-ion transitions. Storage Stability The storage stability of spinosad in final extracts derived from fortified Tenax tubes was measured after 4 and 7 days of storage—both at ambient temperature and at 4 °C. There was no significant loss of analyte observed even after 7 days when stored at either at ambient	

Section A4 (4.2) **Annex Point IIA, IIA-IV.4.2**	**Analytical Methods for Detection and Identification** Specify where appropriate, e.g. isomer of a.s., metabolite of a.s., impurity of a.s., matrix 4.1 for the determination of pure active substance 4.2 **Analytical methods on**: (a) soil; **(b) air**; (c) water; (d) animal and human body fluids and tissues	
	temperature or at 4 °C. The storage stability of spinosad in fortified Tenax adsorbent tubes was determined for tubes stored at <-18 °C, at 4 °C, and at ambient temperature for 4 and 8 days. There was a small loss of the analyte observed after the 4-day storage period at ambient temperature and a more significant loss of the analyte observed in the tubes stored at ambient temperature after the 8-day period. The tubes stored at 4 °C or at <-18 °C did not show any significant loss of analyte after either the 4 or the 8-day storage periods.	
11.2 onclusion	The data summarized below demonstrates the suitability of method GRM 02.18 for the analysis of spinosad residues in air.	
11.2.1 Reliability	1	
11.2.2 Deficiencies	No	

Analyte	Matrix[a]	Fortification Level (µg)	Fortification Level (µg)[b]	Average Recovery (%)	Recovery Range (%)	SD (%)	RSD (%)
Spinosyn A	Air (ambient)	0 264 – 26 4	0 228 – 22 8	85	77 - 94	6 96	8 22
Spinosyn A	Air (elevated)	0 264 – 26 4	0 228 – 22 8	89	76 - 101	6 81	7 61
Spinosyn D	Air (ambient)	0 264 – 26 4	0 0360 – 3 60	86	75 - 96	7 04	8 22
Spinosyn D	Air (elevated)	0 264 – 26 4	0 0360 – 3 60	89	75 - 95	5 50	6 19

[a]Ambient air conditions are approximately 22 °C and 43% relative humidity Elevated air conditions are approximately 35 °C and 80% relative humidity
[b]Fortification based on weight percent of spinosyns A and D – weight percent: 82 3% spinosyn A and 13 0% spinosyn D (overall purity of spinosad was 95 3%)

	Evaluation by Competent Authorities	
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted	
	EVALUATION BY RAPPORTEUR MEMBER STATE	
Date	28-11-2006	
Materials and methods	See Remarks	
Conclusion	See Remarks	
Reliability	See Remarks	
Acceptability	See Remarks	
Remarks	Based on the same studies, the following conclusion was drawn in the second Addendum to the DAR, issued in May/July 2005 and revised in March 2006: Analysis method GRM 02.18 is valid for the determination of spinosyn A and D in air. The method has been validated over the concentration range of 0.73 µg/m^3 to 73 µg/m^3. On the basis of the ADI of 0.024 mg/kg body weight, the required LOQ is 7.2 µg/m^3, so the validated LOQ is sufficient. Storage at room temperature results in significant loss of the analyte. A confirmatory technique is not considered necessary in view of the specific identification used.	
	COMMENTS FROM...	
Date	*Give date of comments submitted*	
Results and discussion	*Discuss additional relevant discrepancies referring to the (sub) heading numbers and to applicant's summary and conclusion.* *Discuss if deviating from view of rapporteur member state*	
Conclusion	*Discuss if deviating from view of rapporteur member state*	
Reliability	*Discuss if deviating from view of rapporteur member state*	
Acceptability	*Discuss if deviating from view of rapporteur member state*	
Remarks		

Section A4.2 Annex Point IIA, IIA-IV.4.2	Analytical Methods for Detection and Identification Specify where appropriate, e.g. isomer of a.s., metabolite of a.s., impurity of a.s., matrix 4.2 **Analytical methods on:** (a) soil; (b) air; **(c) water**; (d) animal and human body fluids and tissues	
	As described in the "Guidance Document on How to utilize PPP dossiers/monograph" of 21 November 2003 the evaluation of the Competent Authority from the PPP monograph/draft assessment report (Vol. 3 / Annex B) is used. The methods have been evaluated in the 91/414/EC Draft Assessment Report (DAR) on Spinosad of February 2001 in section Spinosadvol3B5 – Annex B – PCP and Methods Section. The full 91/414/EEC DAR and its addenda are enclosed in the "other documentation" section as described Document I.1 – Application form, point 6.9. No further information was given in the 1st addendum to the DAR of June 2002.	Official use only

Below is an unchanged copy of the relevant parts of the Spinosad 91/414/EC Draft Assessment Report (DAR) written by the CA (CTB) and released in February 2001 and the 2nd addendum to the DAR of May 2005. Numbering as in the DAR and the Addendum remains unchanged for ease of back tracking.

B.5.3.2 Residues in water (Annex IIA 4.2.3) - Taken from the 91/414/EEC Draft Assessment Report of February 2001

a) OR74

Description

Residues of spinosyns A, D, B and N-demethylated spinosyn D are extracted from water with methyl-tert-butyl ether after addition of 1 M NaOH. An aliquot of the ether layer is evaporated to dryness and reconstituted in the HPLC mobile phase. Spinosyn A, spinosyn D, spinosyn B, spinosyn K, N-demethylated spinosyn D residues are determined simultaneously by reversed phase HPLC with positive ion atmospheric pressure ionisation mass spectroscopy detection (+APCI/MS).

Results

Average recovery values of 95% to 109% resulted from fortified samples (n=32) over four concentrations ranging from 0.1 to 5.0 µg/L for spinosyn A, B, D, K or N-demethylated spinosyn D in drinking water, surface water or ground water. The relative standard deviation (RSD) ranged from 4.1 to 11% for all five analytes at all fortification levels. The average correlation coefficient (r^2) for the least squares regression describing the detector response as a function of concentration (n=8) was 0.95 for all five analytes. The limit of detection was 0.02 µg/L, and the limit of quantification was 0.1 µg/L.

b) OR 17

Description

The method is based upon use of the Strategic Diagnostics Spinosad RaPID™ Assay test kit and the RPA-1 RaPID Analyser. The antibody used in the spinosad immunoassay test kit is sensitive to several spinosyns, including the active ingredients (spinosyns A and D). The kit uses spinosyn A, the major component of spinosad, for generation of the calibration curve and subsequent quantitation of the residue. The method is not designed to differentiate individual spinosyns, but instead measures the total residue of spinosyns and its degradation products.

An aliquot of the water sample is diluted with Spinosad Sample Diluent and then assayed for spinosyn residues using the Strategic Diagnostics Spinosad RaPID Assay test kit, which applies the principles of enzyme-linked immunosorbent assay. An aliquot of the sample is incubated with enzyme-conjugated spinosad and magnetic particles coated with antibodies specific to spinosad. The spinosad in the sample and the enzyme-conjugated spinosad compete for antibody sites on the magnetic particles. At the end of the incubation period, a magnetic field is applied to the particles. The spinosad and enzyme-conjugated spinosad, which are bound to the antibodies on the particles, are held in the sample tube by the magnetic field while the unbound reagents are decanted. The presence of spinosyns is detected by incubating the antibody-bound enzyme conjugate with an enzyme substrate (hydrogen peroxide) and a chromogen (3,3',5,5'-tetramethylbenzidine), generating a coloured product. Since the enzyme-labelled spinosyns are in competition with free (sample) spinosyns for the antibody sites, the level of colour development is inversely proportional to the concentration of the spinosyns in the sample (i.e., lower residue concentrations result in greater colour development). The absorbance at 450 nm is measured in each sample tube using the RPA-1 RaPID Analyser. A calibration curve is generated and the spinosyn concentration in unknown samples is calculated from the regression equation using the pre-programmed software capabilities of the RPA-1 RaPID Analyser.

Results

Average recovery values ranging from 91-112% resulted from fortified samples (n=31) over six concentrations ranging from 0.1 to 20.0 µg/L for spinosad. The relative standard deviation (RSD) ranged from 3.8% to 14%. The limit of detection was 0.042 µg/L, and the limit of quantification was 0.14 µg/L.

B.5.5 EVALUATION AND ASSESSMENT

Analytical methods (residue) soil, water, air

Where information has been submitted, it fulfils the following criteria:

adequate limit of determination (soil: 0.05 mg/kg, water: 0.1 µg/L);

mean recovery 70-110%;

relative standard deviation of recovery rates <20%;

interfering blanks lower than 30% of the limit of determination;

readily available equipment and reagents used.

In Table B.5.5-1 the method descriptions and validation data are summarised.

Table B.5.5-1 Summary of method description and validation

Substrate	Procedure	Analyte	LOQ	Recovery fortification level	Recoveries range (mean) [%]	Repeatability RSD (n) [%]	Linearity demonstrated	Reference
drinking water	Extraction with methyl-tert-butyl ether, analysis by HPLC with positive ion atmospheric pressure ionisation mass spectroscopy detection (APCI/MS)	spinosyn A spinosyn D spinosyn B N-demethylated spinosyn D spinosyn K	0.1 µg/L	0.1-5.0 µg/L	99-115 (107) 87-118 (105) 91-122 (109) 87-121 (107) 100-117 (108)	4.7 (n=32) 8.1 (n=32) 7.3 (n=32) 7.9 (n=32) 4.1 (n=32)	yes	OR74
surface water		spinosyn A spinosyn D spinosyn B N-demethylated spinosyn D spinosyn K			80-111 (98) 84-117 (99) 89-118 (102) 88-119 (101) 91-114 (100)	6.3 (n=32) 7.9 (n=32) 6.6 (n=32) 7.2 (n=32) 5.4 (n=32)		
ground water		spinosyn A spinosyn D spinosyn B N-demethylated spinosyn D spinosyn K			73-101 (95) 64-109 (96) 80-108 (100) 73-108 (99) 86-108 (98)	6.4 (n=32) 11 (n=32) 8.1 (n=32) 8.6 (n=32) 4.7 (n=32)		
water	An aliquot of the water sample is diluted with Spinosad Sample Diluent and incubated with enzyme-conjugated spinosad and magnetic particles coated with antibodies specific to spinosad. The spinosad in the sample and the enzyme-conjugated spinosad compete for antibody sites on the magnetic particles. At the end of the incubation period, a magnetic field is applied to the particles, keeping the spinosad and enzyme-conjugated spinosad that are bound to the antibodies on the particles in the sample tube, while the unbound reagents are decanted. The antibody-bound enzyme conjugate is incubated with an enzyme substrate (hydrogen peroxide) and a chromogen (3,3',5,5'-tetramethylbenzidine), generating a coloured product. The absorbance at 450 nm is measured in each sample tube using the RPA-1 RaPID Analyser	several individual spinosyns as well as some metabolites the method is not capable of differentiating individual spinosyns	0.14 µg/L	0.1-20.0 µg/L	71-123 (100)	12 (n=61)	NA	OR17

B.5.6 References relied on

91/414 Annex point / reference no. No.	Author(s)	Year	Title Company Report No. Source (where different from company) GLP or GEP status Published or not	Data protection claimed Y/N	Owner
IIA 4.2.3	Boothroyd, S., Hastings, M. & Drossopoulos, M.	1999	Determination of Residues of Spinosad and its Metabolites in ground Water, Surface Water, and Drinking Water by Performance Liquid Chromatography with Mass Spectrometry Detection. Dow AgroSciences, Report No. ERC 98.23 Ref. OR74 GLP Study Unpublished	Y	DAS (OR74)
IIA 4.2.3	Mihaliak, C.A & Young, D.L.	1995	Determination of Residues of Spinosad in Water Using a Magnetic Particle-Based Immunoassay Test Kit. DowElanco, Report No. GRM 94.10 Ref. OR17 GLP Study Unpublished	Y	DAS (OR17)

B.5.3.2 Residues in water (Annex IIA 4.2.3) - Taken from the 91/414/EEC 2nd Addendum to the Draft Assessment Report of May 2005

Study OR93

Reference/notifier : Rutherford, L.A.
Type of study : analytical method water
Year of execution : 2003
Test substance : spinosyn A
spinosyn B
spinosyn D
N-demethyl spinosyn D
13,14 Beta-dihydro C17 pseudoaglycone of spinosyn A and D

GLP statement : yes
Guideline : not applicable
Acceptability : acceptable

Substrate	Analyte	LOQ [µg/L]	Recovery fortification level [mg/L]	Recoveries: range (mean) [%]	Repeatability RSD (n) [%]	Linearity of response (r^2)
drinking water	spinosyn A	0.01	0.01	71-86 (78)	7 (6)	>0.9988
			0.10	71-79 (75)	4 (6)	
			1.0	81-84 (83)	1 (6)	
	spinosyn B	0.01	0.01	86-99 (94)	5 (6)	>0.9988
			0.10	83-94 (88)	4 (6)	
			1.0	81-87 (85)	3 (6)	
	spinosyn D	0.01	0.01	73-84 (77)	9 (6)	>0.9988
			0.10	69-77 (72)	4 (6)	
			1.0	77-82 (79)	3 (6)	
	N-demethyl spinosyn D	0.01	0.01	86-105 (95)	9 (6)	>0.9988
			0.10	85-96 (89)	4 (6)	
			1.0	86-90 (88)	2 (6)	
	13,14 Beta-dihydro C17 pseudoaglycone of spinosyn A	0.01	0.01	88-107 (99)	6 (6)	>0.9988
			0.10	90-96 (94)	2 (6)	
			1.0	91-95 (93)	2 (6)	
	13,14 Beta-dihydro C17 pseudoaglycone of spinosyn D	0.01	0.01	83-102 (92)	9 (6)	>0.9988
			0.10	86-92 (89)	2 (6)	
			1.0	87-97 (91)	5 (6)	
ground water	spinosyn A	0.01	0.01	77-85 (80)	4 (6)	>0.9988
			0.10	72-81 (77)	4 (6)	
			1.0	79-89 (84)	5 (6)	
	spinosyn B	0.01	0.01	83-94 (88)	4 (6)	>0.9988
			0.10	89-91 (90)	1 (6)	
			1.0	83-90 (87)	4 (6)	
	spinosyn D	0.01	0.01	72-75 (74)	1 (6)	>0.9988
			0.10	67-77 (73)	6 (6)	
			1.0	72-86 (78)	8 (6)	
	N-demethyl spinosyn D	0.01	0.01	91-97 (94)	3 (6)	>0.9988
			0.10	91-96 (93)	2 (6)	
			1.0	86-93 (89)	3 (6)	
	13,14 Beta-dihydro C17 pseudoaglycone of spinosyn A	0.01	0.01	92-102 (96)	4 (6)	>0.9988
			0.10	93-99 (96)	2 (6)	
			1.0	93-97 (95)	2 (6)	
	13,14 Beta-dihydro C17 pseudoaglycone of spinosyn D	0.01	0.01	83-99 (92)	8 (6)	>0.9988
			0.10	89-96 (93)	3 (6)	
			1.0	89-93 (91)	2 (6)	
surface water	spinosyn A	0.01	0.01	77-96 (87)	9 (6)	>0.9988
			0.10	75-93 (86)	8 (6)	
			1.0	84-91 (88)	3 (6)	
	spinosyn B	0.01	0.01	87-101 (95)	6 (6)	>0.9988
			0.10	88-97 (93)	4 (6)	
			1.0	84-88 (86)	2 (6)	
	spinosyn D	0.01	0.01	74-95 (84)	9 (6)	>0.9988
			0.10	81-90 (86)	4 (6)	
			1.0	82-86 (84)	2 (6)	
	N-demethyl spinosyn D	0.01	0.01	89-112 (97)	9 (6)	>0.9988
			0.10	86-102 (94)	6 (6)	
			1.0	84-87 (85)	2 (6)	
	13,14 Beta-dihydro C17 pseudoaglycone of spinosyn A	0.01	0.01	85-102 (94)	7 (6)	>0.9988
			0.10	91-99 (96)	4 (6)	
			1.0	90-99 (93)	3 (6)	
	13,14 Beta-dihydro C17 pseudoaglycone of spinosyn D	0.01	0.01	89-96 (92)	3 (6)	>0.9988
			0.10	92-97 (94)	2 (6)	
			1.0	85-93 (88)	3 (6)	

Description

Method validation.
Three types of water were applied with a stock of mixed spinosyn A, D, B and N-demethylated spinosyn D and a stock of mixed 13,14 beta-dihydro C17-pseudoaglycone of spinosyn A and D. Fortification levels 0.003- 1.0 µg/L with 6 replicates each, a reagent blank and a control.
Analysis method.
GRM 03.17. Residues of spinosad and its metabolites in water samples are extracted using methyl *tert*-butyl ether and concentrated under nitrogen. The residues are reconstituted in an acetonitrile/water (40:60) solution containing 5 mM ammonium acetate. The final solution is analysed by LC/MS/MS. The presence of spinosad and its metabolites is confirmed by comparing the liquid chromatography retention times of the analyte in the calibration standards with those found in the samples as well as by the MS/MS transitions monitored.

Results

LOQ 0.01 µg/L defined as lowest fortification level with acceptable recovery. A calibration curve resulting from the injection of eight standards demonstrated linearity with a correlation coefficient of at least 0.9988.

Remarks by RMS

No information available on characteristics of water used in the study. Purity of test substances not reported. Method meets validity criteria.

B.5.6 References relied on

91/414/EEC Annex point/ reference no.	Author(s)	Year	Title Company, report no. Source (where different from company) GLP or GEP status (where relevant) Published or not	Data Protection Claimed Y/N	Owner
IIA, 4.2.3	Rutherford, L.A., Hastings, M.J.	2003	Determination of residues of spinosad and its metabolites in drinking water, ground water, and surface water by Liquid Chromatography with Tandem Mass Spectrometry	Y	DAS (OR 93)

Section A4 (4.2) **Annex Point IIA, IIA-IV.4.2**	**Analytical Methods for Detection and Identification** Specify where appropriate, e.g. isomer of a.s., metabolite of a.s., impurity of a.s., matrix 4.1 for the determination of pure active substance 4.2 **Analytical methods on:** (a) soil; (b) air; **(c) water**; (d) animal and human body fluids and tissues	
	12 REFERENCE (REF. A4.2/03, OR93)	**Official use only**
12.1 Reference	Rutherford, L. A. and Hastings, M. J., 2003, Determination of Residues of Spinosad and its Metabolites in Drinking Water, Ground Water, and Surface Water by Liquid Chromatography with Tandem Mass Spectrometry. Regulatory Laboratories, Indianapolis, Indiana, USA. (12-June-2003) (Dow AgroSciences Method GRM 03.17). **(Ref. A4.2/03, OR93)**	
12.2 Data protection	Yes	
12.2.1 Data owner	Dow AgroSciences LLC	
12.2.2 Companies with letter of access	none	
12.2.3 Criteria for data protection	Data on new active substance for first entry to Annex I authorisation	
	13 GUIDELINES AND QUALITY ASSURANCE	
13.1 Guideline study	Yes	
13.2 GLP	Yes	
13.3 Deviations	No	
	14 MATERIALS AND METHODS	
14.1 Preliminary treatment		
14.1.1 Enrichment	Residues of spinosad and it metabolites are extracted from a 10-mL water sample under alkaline conditions by partitioning with methyl *tert*-butyl ether (MTBE) and concentrated under nitrogen.	
14.1.2 Cleanup	The residuum is reconstituted with an acetonitrile/water (40:60) solution containing 5 mM ammonium acetate.	
14.2 Detection		
14.2.1 Separation method	The samples are chromatographed by gradient high performance liquid chromatography using a Luna Phenyl-Hexyl column (5-µm, 50 x 2.00 mm i.d.) with a acetonitrile:water:ammonium acetate mobile phase.	
14.2.2 Detector	Detection of spinosad and its metabolite residues is performed by positive-ion electrospray ionization (ESI) tandem mass spectrometry (MS/MS) monitoring the analyte specific precusor-ion/product ion transitions (fragments) of the spinosyns as follows: Spinosyn A *m/z* Q1/Q3 732.5/142.1 Spinosyn D *m/z* Q1/Q3 746.5/142.1 Spinosyn B *m/z* Q1/Q3 718.5/128.1 *N*-demethyl spinosyn D *m/z* Q1/Q3 732.5/128.1	

Section A4 (4.2) **Annex Point IIA, IIA-IV.4.2**	**Analytical Methods for Detection and Identification** Specify where appropriate, e.g. isomer of a.s., metabolite of a.s., impurity of a.s., matrix 4.1 for the determination of pure active substance 4.2 **Analytical methods on:** (a) soil; (b) air; **(c) water**; (d) animal and human body fluids and tissues	
	β-13,14-dihydro C17-pseudoaglycone of Spinosyn A *m/z* Q1/Q3 610.4/189.1 β-13,14-dihydro C17-pseudoaglycone of Spinosyn D *m/z* Q1/Q3 624.5/189.1	
14.2.3 Standard(s)	Quantitation of residues of spinosad and its metabolite is performed using an external calibration standard technique.	
14.2.4 Interfering substance(s)	During the validation study, 7 different water samples were analysed. No interferences from co-extracted species were observed.	
14.3 Linearity		
14.3.1 Calibration range	0.00001 – 0.0050 µg/mL (0.01 – 5.0 ng/mL). Equivalent to 0.0010 – 0.50 ng/mL (µg/L) spinosyns A and D and their metabolites, spinosyn B, N-demethyl spinosyn D, 13,14β-dihydro C17-pseudoaglycone of Spinosyn A (referred to as spinosyn A pseudoaglycone in this method), and 13,14β-dihydro C17-pseudoaglycone of Spinosyn D (referred to as spinosyn D pseudoaglycone in this method), in drinking water, ground water, and surface water.	
14.3.2 Number of measurements	8 standards injected throughout the analytical run.	
14.3.3 Linearity	The calibration curves from 3 analytical runs yielded correlation coefficients (r) of at least 0.9988.	

Section A4 (4.2)

Annex Point IIA, IIA-IV.4.2

Analytical Methods for Detection and Identification

Specify where appropriate, e.g. isomer of a.s., metabolite of a.s., impurity of a.s., matrix

4.1 for the determination of pure active substance

4.2 **Analytical methods on:** (a) soil; (b) air; **(c) water**; (d) animal and human body fluids and tissues

14.4 Specifity: interfering substances

HPLC/MS/MS affords a highly specific method for both quantitation and confirmation of residue identity by retention time matching in conjunction with monitoring the specific MS/MS ion transitions of spinosad and its metabolites.

14.5 Recovery rates at different levels

Analyte	Fortification Level (µg/L)	Average Recovery (%)	Recovery Range (%)	n
Spinosyn A	0.01	82	71 - 96	18
	0.1	79	71 - 93	18
	1.0	85	79 - 91	18
	0.01 – 1.0	82	71 - 96	54
Spinosyn D	0.01	78	68 - 95	18
	0.1	77	67 - 90	18
	1.0	81	72 - 86	18
	0.01 – 1.0	79	67 - 95	54
Spinosyn B	0.01	92	83 - 101	18
	0.1	90	83 - 97	18
	1.0	86	81 - 90	18
	0.01 – 1.0	89	81 - 101	54
N-demethyl Spinosyn D	0.01	95	86 - 112	18
	0.1	92	85 - 102	18
	1.0	87	84 - 93	18
	0.01 – 1.0	92	84 - 112	54
β-13,14-dihydro C17-pseudoaglycone Of Spinosyn A	0.01	96	85 - 107	18
	0.1	95	90 - 99	18
	1.0	94	90 - 99	18
	0.01 – 1.0	95	85 - 107	54
β-13,14-dihydro C17-pseudoaglycone of Spinosyn D	0.01	92	83 - 102	18
	0.1	92	86 - 97	18
	1.0	90	85 - 97	18
	0.01 – 1.0	91	83 - 102	54

Section A4 (4.2) **Annex Point IIA, IIA-IV.4.2**	**Analytical Methods for Detection and Identification** Specify where appropriate, e.g. isomer of a.s., metabolite of a.s., impurity of a.s., matrix 4.1 for the determination of pure active substance 4.2 **Analytical methods on:** (a) soil; (b) air; **(c) water**; (d) animal and human body fluids and tissues	

14.5.1 Relative standard deviation

Analyte	Fortification Level (µg/L)	SD (%)	RSD (%)	n
Spinosyn A	0.01	7	8	18
	0.1	7	9	18
	1.0	3	4	18
	0.01 – 1.0	6	8	54
Spinosyn D	0.01	7	9	18
	0.1	7	9	18
	1.0	5	6	18
	0.01 – 1.0	7	8	54
Spinosyn B	0.01	5	6	18
	0.1	4	4	18
	1.0	2	3	18
	0.01 – 1.0	5	5	54
N-demethyl Spinosyn D	0.01	7	7	18
	0.1	5	5	18
	1.0	2	3	18
	0.01 – 1.0	6	6	54
β-13,14-dihydro C17-pseudoaglycone Of Spinosyn A	0.01	6	6	18
	0.1	3	3	18
	1.0	2	2	18
	0.01 – 1.0	4	4	54
β-13,14-dihydro C17-pseudoaglycone of Spinosyn D	0.01	6	7	18
	0.1	3	3	18
	1.0	3	3	18
	0.01 – 1.0	4	5	54

Section A4 (4.2) **Annex Point IIA, IIA-IV.4.2**	**Analytical Methods for Detection and Identification** Specify where appropriate, e.g. isomer of a.s., metabolite of a.s., impurity of a.s., matrix 4.1 for the determination of pure active substance 4.2 **Analytical methods on:** (a) soil; (b) air; **(c) water**; (d) animal and human body fluids and tissues	
14.6 Limit of determination	Following established guidelines (1), the limits of quantitation (LOQ) and detection (LOD) for the determination of spinosad and its metbolites in water samples were calculated using the standard deviation of the 0.01-µg/L (ng/mL) recovery results. The LOQ was calculated as ten times the standard deviation (10s), and the LOD was calculated as three times the standard deviation (3s) of the results of the analysis of a minimum of 18 samples. The results are summarized below.	
14.7 Precision		
14.7.1 Repeatability	No specific repeatability data was generated. However, the data presented in section 3.5, 3.5.1, and 3.6 is a composite of three analytical validation batches generated over a period of five days.	
14.7.2 Independent laboratory validation	No independent validation was conducted on method GRM 03.17.	

Analyte	Average Recovery, (µg/L)	Standard Deviation, (*s*)	Limit of Detection, (3*s*)	Limit of Quantitation, (10*s*)
Spinosyn A	0 00818	0 00068	0 0020	0 0068
Spinosyn D	0 00781	0 00072	0 0022	0 0072
Spinosyn B	0 00923	0 00053	0 0016	0 0053
N-demethyl Spinosyn D	0 00953	0 00067	0 0020	0 0067
13,14β-dihydro C17-pseudoaglycone of Spinosyn A	0 00962	0 00058	0 0017	0 0058
13,14β-dihydro C17-pseudoaglycone of Spinosyn D	0 00919	0 00060	0 0018	0 0060

The calculated LOQ supports the validated method LOQ of 0.01 ng/mL. The calculated LOD supports the validated method LOD of 0.003 ng/mL.

(1) *Keith, L. H.; Crummett, W.; Deegan, J., Jr.; Libby, R. A.; Taylor, J. K.; Wentler, G.* Anal. Chem. ***1983***, 55, *2210-2218.*

Section A4 (4.2) **Annex Point IIA, IIA-IV.4.2**	**Analytical Methods for Detection and Identification** Specify where appropriate, e.g. isomer of a.s., metabolite of a.s., impurity of a.s., matrix 4.1 for the determination of pure active substance 4.2 **Analytical methods on:** (a) soil; (b) air; **(c) water**; (d) animal and human body fluids and tissues	
	15 APPLICANT'S SUMMARY AND CONCLUSION	
15.1 Materials and methods	Dow AgroSciences method GRM 03.11 is applicable for the quantitative determination of residues of spinosad and its metbolites in water. The method was validated over the concentration range of 0.01-1.0 µg/L (ng/mL). The validated limit of quantitation for the method is 0.01 µg/L (ng/mL). Residues of spinosad and it metabolites are extracted from a 10-mL water sample under alkaline conditions by partitioning with methyl *tert*-butyl ether (MTBE) and concentrated under nitrogen. The residuum is reconstituted with an acetonitrile/water (40:60) solution containing 5 mM ammonium acetate. Detection of spinosad and its metabolite residues is performed by positive-ion electrospray ionization (ESI) tandem mass spectrometry (LC/MS/MS). A calibration curve resulting from the injection of eight standards demonstrated linearity with a correlation coefficient of at least 0.9988. LC/MS/MS affords a highly specific method for quantitation and confirmation of spinosad and its metabolites by retention time matching with standards in conjunction with monitoring analyte specific precursor-ion/product-ion transitions.	
15.2 Conclusion	The data summarized below demostrates the suitability of method GRM 03.17 for the anlysis of spinosad and its metbolite residues in water samples.	
15.2.1 Reliability	1	
15.2.2 Deficiencies	No	

Analyte	Fortification Level, (µg/L)	Average Recovery (%)	Recovery Range (%)	SD (%)	RSD (%)
Spinosyn A	0.01 – 1.0	82	71 - 96	6	8
Spinosyn D	0.01 – 1.0	79	67 - 95	7	8
Spinosyn B	0.01 – 1.0	89	81 - 101	5	5
N-demethyl spinosyn D	0.01 – 1.0	92	84 - 112	6	6
13,14β-dihydro C17-pseudoaglycone of Spinosyn A	0.01 – 1.0	95	85 - 107	4	4
13,14β-dihydro C17-pseudoaglycone of Spinosyn D	0.01 – 1.0	91	83 - 102	4	5

	Evaluation by Competent Authorities	
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted	
	EVALUATION BY RAPPORTEUR MEMBER STATE	
Date	28-11-2006	
Materials and methods	See Remarks	
Conclusion	See Remarks	
Reliability	See Remarks	
Acceptability	See Remarks	
Remarks	Based on the same studies, the following conclusion was drawn in the second Addendum to the DAR, issued in May/July 2005 and revised in March 2006: Analysis method GRM 03.17 is valid for the determination of spinosyn A and D, and their metabolites, spinosy B, N-demethyl spinosyn D, 13,14beta-dihydro-C17-pseudoaglycone of Spinosyn A and D in drinking water, ground water, and surface water. The method was validated over the concentration range of 0.01-1.0 µg/L with a validated LOQ of 0.01 µg/L. A confirmatory technique is not considered necessary in view of the specific identification method used.	
	COMMENTS FROM...	
Date	*Give date of comments submitted*	
Results and discussion	*Discuss additional relevant discrepancies referring to the (sub) heading numbers and to applicant's summary and conclusion.* *Discuss if deviating from view of rapporteur member state*	
Conclusion	*Discuss if deviating from view of rapporteur member state*	
Reliability	*Discuss if deviating from view of rapporteur member state*	
Acceptability	*Discuss if deviating from view of rapporteur member state*	
Remarks		

Section A4.2 **Annex Point IIA, IIA-IV.4.2**	**Analytical Methods for Detection and Identification** Specify where appropriate, e.g. isomer of a.s., metabolite of a.s., impurity of a.s., matrix 4.2 **Analytical methods on:** (a) soil; (b) air; (c) water; **(d) animal and human body fluids and tissues**	
	Spinosad is not classified as toxic or highly toxic, therefore according to the Technical Notes on Guidance, Chapter 2, Part A, no methods on animal and human body fluids and tissues need to be submitted. However, methods of spinosad in human plasma and urine have already been evaluated for the 91/414/EEC submission and are included.	**Official use only**
	As described in the "Guidance Document on How to utilize PPP dossiers/monograph" of 21 November 2003 the evaluation of the CA from the PPP monograph (Vol. 3 / Annex B) is used. The methods have been evaluated in the 91/414/EC Draft Assessment Report on Spinosad of February 2001 in section Spinosadvol3B5 – Annex B – PCP and Methods Section. No further information is given in the addendum of June 2002 and of May 2005. The full 91/414/EEC DAR and its addenda are enclosed in the "other documentation" section as described Document I.1 – Application form, point 6.9.	

Below is an unchanged copy of the relevant parts of the 91/414/EEC Spinosad Draft Assessment Report (DAR) written by the CA (CTB) and released in February 2001. Numbering as in the DAR remains unchanged.

B.5.4 Analytical methods (residue) for body fluids and tissues (Annex IIA 4.2.5; Annex IIIA 5.2)

Method for the determination of Spinosad in human plasma and urine

Residues of spinosad (spinosyns A and D) were determined with reversed phase HPLC with $^{13}C,D_3$ stable isotopes of factors A and D as internal standards.

Residues were determined in urine after filtration with a 0.2 μm filter and in human plasma after mixing with an equal volume acetonitrile followed by centrifugation at 300 rpm.

Quantification is made by HPLC with positive ionization-mass spectrometry (+ESI/MS) detection.

(OR76, Markham et al, 1999)

B.5.5 EVALUATION AND ASSESSMENT

Analytical methods (residue) for body fluids and tissues

No analytical methods were submitted for the determination of spinosad residues in animal products. In view of the fact that no residue intake by livestock animals is expected, no residue definition is proposed and thus no analytical methods for animal products are required.

B.5.6 References relied on

91/414 Annex point / reference no. No.	Author(s)	Year	Title Company Report No. Source (where different from company) GLP or GEP status Published or not	Data protection claimed Y/N	Owner
IIA 4.2.5	Markham, D.A. and Bartels, M.J.	1999	Analytical Method Validation for the Determination of Spinosad Factors A and D in Human Plasma and Urine. The Dow Chemical Company, Report No. 981205 Ref. OR76 GLP Study Unpublished	Y	DAS

	Evaluation by Competent Authorities	
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted	
	EVALUATION BY RAPPORTEUR MEMBER STATE	
Date	27 November 2006	
Materials and methods	No comments.	
Conclusion	No comments	
Reliability	-	
Acceptability	acceptable	
Remarks	-	
	COMMENTS FROM...	
Date	*Give date of comments submitted*	
Results and discussion	*Discuss additional relevant discrepancies referring to the (sub) heading numbers and to applicant's summary and conclusion.* *Discuss if deviating from view of rapporteur member state*	
Conclusion	*Discuss if deviating from view of rapporteur member state*	
Reliability	*Discuss if deviating from view of rapporteur member state*	
Acceptability	*Discuss if deviating from view of rapporteur member state*	
Remarks		

Section IIA 4.3 Annex Point IIIA, IV.1	Analytical Methods for the active substance and residues thereof in food or feedstuffs	
	JUSTIFICATION FOR NON-SUBMISSION OF DATA	Official use only
Other existing data [ ]	**Technically not feasible [ ]** **Scientifically unjustified [ ]**	
Limited exposure [x]	**Other justification [ ]**	
Detailed justification:	The active ingredient is used as a biocide in a fly bait formulation for control of the housefly *(Musca domestica)* indoors in animal stables. The flybait will not be used on any food or feedingsstuff. Even though many methods have been developed for the 91/414/EC PPP use of spinosad in crops, these are not relevant for the biocidal application and are thus not submitted.	
Undertaking of intended data submission [ ]		

	Evaluation by Competent Authorities	
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted	
	EVALUATION BY RAPPORTEUR MEMBER STATE	
Date	27 November 2006	
Evaluation of applicant's justification	The active ingredient is used as biocide for control of housefly indoors in animal stables. Spinosad residues in foodstuffs of animal origin can be expected if livestock consumes the biocidical product, when this is scattered, sprayed or painted onto a surface within reach of the livestock or when livestock consumes feed sprayed with the biocidal product. The presence of spinosad residues should therefore be verified in food or feed from animal origin. Analytical methods for food or feed from animal origin have been submitted in document IIIB and are evaluated there.	
Conclusion	No analytical methods are required for the determination of spinosad residues in food and feed from plant origin. Satisfactory analytical methodology for food/feed of animal origin is summarised in doc IIIB.	
Remarks	-	
	COMMENTS FROM OTHER MEMBER STATE *(specify)*	
Date	*Give date of comments submitted*	
Evaluation of applicant's justification	*Discuss if deviating from view of rapporteur member state*	
Conclusion	*Discuss if deviating from view of rapporteur member state*	
Remarks		